PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES AND STEM OF *Ipomoea sepiaria koen. ex Roxb.*

Dissertation submitted to
The Tamilnadu Dr. M.G.R. Medical University,
Chennai
In partial fulfillment of the requirement for the
Degree of

MASTER OF PHARMACY
IN
PHARMACOGNOSY
Submitted
BY
26108670

MAY – 2012

DEPARTMENT OF PHARMACOGNOSY
MADURAI MEDICAL COLLEGE
MADURAI – 625020

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ACKNOWLEDGEMENT

It is my privilege and honour to express my sincere thanks to our respectful sir **Dr. Edwin Joe M.D.,** Dean, Madurai Medical College, Madurai and **Dr.T. Meena M.D**.Vice Principal, Madurai Medical College, Madurai for providing me with all the necessary facilities to do my project work.

I am very thankful to **Dr. Mrs. Ajithadas Aruna, M.Pharm., Ph.D** Principals, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai for her excellent encouragement, guidance, boundless enthusiasm, motivation and valuable advice for the successful completion of my project.

I wish to place on record here my indebtedness and heartfelt thanks to **Mr.T.Venkatarathinakumar M.Pharm., (Ph.D)** Assistant Reader, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai for his enthusiastic co-operation as my project guide & for all the constant valuable suggestions and encouragement to improve and complete the project work.

I extend my special and sincere thanks to **Ms.R.Gowri, M.Pharm,** Assistant Reader, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai for her diligence and for all the consistent encouragement, suggestions, contribution and support extended during the project work.

I express my sincere thanks to **Dr. Mr. k. Periyanayagam M.Pharm., Ph.D** Assistant Reader, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai for his friendly and cheerful guidance during the course.

I am thankful to **Dr.Mr. Jonat M.V.Sc.** Assistant Surgeon, Central Animal House, Madurai Medical College, Madurai, for their constant support for permitting me to utilize animals for pharmacological study of this dissertation.

I owe my special thanks to **Mr.Arunachalam Muthuraman M.Pharm (Ph.D)** Department of Pharmaceutical Science and Drug Research, Punjab University, whose valuable suggestion regarding the pharmacological studies and timely help made this work as a successful one.

I am thankful to **Mrs.A.Sedhuramani M.Pharm (Ph.D)** and **Mrs.A.Krishnaveni. M.Pharm (Ph.D)** Tutors in Pharmacognosy, Madurai Medical College, Madurai for their friendly and encouragement to improve and complete this work.

I extend my special thanks to **Miss.G. Shanthini nachiar** M.pharm,department of pharmacognosy,college of pharmacy, Madurai medical college Madurai.

I extend my special thanks to **Prof. Dr.P. Jayaraman, Ph.D,** Director (PARC) Chennai for his help regarding the Microscopy and authentification of the plant.

I extend my special and sincere thanks to **Dr.Mr.Sethuraman M.phill, Phd, ASSISSTANT MANAGER IN BAL pharma pvt ltd**, his diligence and for all the consistent encouragement, suggestions, contribution and support extended during the project work.

I express my gratitude to **Mr.M.BARANI ,Msc** Assisstant manager in **MEDRICH** pharma pvt ltd Bangalore for his support and help in carry out the analytical calculations of this work.

I place on record my sincere thanks to **Mr.Sureshkumar B.pharm, MBA**,R&D in ORCHID ltd Irunnkaatukotai chennai for hiscontinuous support on thesystem applications at appropriate timings

I express my sincere thanks to **Mr. N. Cheransenguttuvan** for his generous help in accomplishing this scientific work.

I thank **Mr.P.Sivakumar M.sc** Lab supervisor and **Mrs S.Kalpana sivakumar** M.Sc. Jayanithi uro labs I am thankful to **Mr. Johns** Universal Scientific Suppliers for his timely supply of chemicals to carry out this work.

I am thankful to my juniors for their help for this project work.

I also thank the faculty and non faculty of the pharmacognosy department for their help during the course of my dissertation work.

Most of all I would like to thank my lovable , mother,sisters and their family members all the support and love they have given to me, always they are the reason behind my every successful moments.

CHAPTER-I

INTRODUCTION

Medicinal and aromatic plants have been used since ancient times in the traditional practices for health care. it is well known that plants are an important source for a variety of bioactive compounds for a variety of purposes including pharmacy ,medicine and industry, According to WHO report nearly 70-80% of the world population relies on traditional medicine largely which are produces plant based for primary health care needs. the annual international market of herbal product is estimated to be US$20 million. with increasing popular demand for medicinal plants both in india and internationally.

Human being had depended only on natural sources to a larger extent particularly on plants of his vicinity for getting rid of these ailments.

Besides for his other needs Several potent medicinal plants have seen the light of the day due to his continous usage of the effective drugs, Some of these plants have attracted the herbalist who have put these plants in to use for centuries together and included the more potent one in the materia medica of their region.

Scientific study of folk medicine is very essential because the efficacy of a drug depends upon various factors such as genuinity, preparation administration and dosage of drugs.

Pharmacognosy is the study of crude drugs obtained from plants, animals and mineral kingdom and their constituents. Even though science of Pharmacognosy is practiced since a very early period.

The term Pharmacognosy was first used by **C.A. SEYDLER,** a German Scientist, in 1865 in his book Analecta Pharmacognostica. It is derived from two Latin words Pharmakon, "a drug" and gignosco, "to acquire knowledge of". It means knowledge or science of drugs.[1,2,3,4,5]

NEUROPATHY

A classical term for any disorders affecting any segment of the nervous system.

NEUROPATHIC PAIN

Neuropathy is a collection of disorders that occurs when nerves of the peripheral nervous system (the part of the nervous system outside of the brain and spinal cord) are damaged. The condition is generally referred to as **peripheral neuropathy**, and it is most commonly due to damage of nerve axons. Neuropathy usually causes pain and numbness in the hands and feet. It can result from traumatic injuries, infections, metabolic disorders, and exposure to toxins.[79, 80]

Neuropathy can affect nerves that control muscle movement (motor nerves) and those that detect sensations such as coldness or pain (sensory nerves). In some cases - autonomic neuropathy - it can affect internal organs, such as the heart, blood vessels, bladder, or intestines. Pain from peripheral neuropathy is often described as a tingling or burning sensation. There is no specific length of time that the pain exists, but symptoms often improve with time - especially if the neuropathy has an underlying condition that can be cured. The condition is often associated with poor nutrition, a number of diseases, and pressure or trauma, but many cases have no known reason and other wise called as idiopathic neuropathy.

NEUROPATHY CLASSIFICATION

Peripheral neuropathy can be broadly classified into the following categories:

- Mononeuropathy - involvement of a single nerve. Examples include carpal tunnel syndrome, ulnar nerve palsy, radial nerve palsy, and peroneal nerve palsy.
- Multiple mononeuropathy - two or more nerves individually affected.
- Polyneuropathy - generalized involvement of peripheral nerves. Examples include diabetic neuropathy and Guillain-Barre syndrome.

Neurophathies may also be categorized based on a functional classification (motor, sensory, autonomic, or mixed) or the type of onset (acute - hours or days, subacute - weeks or months, or chronic - months or years).The most common form of neuropathy is (symmetrical) peripheral polyneuropathy, which mainly affects the feet and legs on both sides of the bod**y.**

CAUSES OF NEUROPATHY

About 30% of neuropathy cases are considered idiopathic, which means they are of unknown cause. Another 30% of neuropathies are due to diabetes. In fact, about 50% of people with diabetes develop some type of neuropathy. The remaining cases of neuropathy, called acquired neuropathies, have several possible causes, including

- Trauma or pressure on nerves, often from a cast or crutch or repetitive motion such as typing on a keyboard.
- Nutritional problems and vitamin deficiencies, often from a lack of B vitamins.

- Alcoholism, often through poor dietary habits and vitamin deficiencies.
- Autoimmune diseases, such as lupus, rheumatoid arthritis, and Guillain-Barre syndrome.
- Tumors, which often press up against nerves.
- Other diseases and infections, such as kidney disease, liver disease, Lymph disease, HIV/AIDS, or an underactive thyroid (hypothyroidism).
- Inherited disorders (hereditary neuropathies), such as Charcot-Marie-Tooth disease and amyloid polyneuropathy.
- Poison exposure, from toxins such as heavy metals, and certain medications and cancer treatments.

WHO GETS NEUROPATHY?

Risk factors for peripheral neuropathy include several conditions and behaviors. People with diabetes who poorly control their blood sugar levels are very likely to suffer from some neuropathy. Autoimmune diseases such as lupus and rheumatoid arthritis also increase one's chance of developing a neuropathy. People who have received organ transplants, AIDS patients, and others who have had some type of immune system suppression have a higher risk of neuropathy. In addition, those who abuse alcohol or have vitamin deficiencies (especially B vitamins) are at an increased risk. Neuropathy is also more likely to occur in people with kidney, liver or thyroid disorders.

SYMPTOMS OF NEUROPATHY

Neuropathy symptoms depend on several factors, chiefly where the affected nerves are located and which type of nerves are affected (motor, sensory, autonomic).

Several types of neuropathy affect all three types of nerves. Some neuropathies suddenly arise while others come on gradually over the course of years.

Motor nerve damage usually leads to symptoms that affect muscles such as muscle weakness, cramps, and spasms. It is not uncommon for this type of neuropathy to lead to a loss of balance and coordination. Patients may find it difficult to walk or Peripheral neuropathic pain has been frequently observed in patients with cancer,AIDS,longstanding diabetes,lumbar disc syndrome,herpes infection,traumatic spinal cord injury,multiple sclerosis and stroke. Moreover,post thoracotomy,post-herniorrhaphy,post-mastectomy,and post-sternotomy have also been associated with neuropathic pain.

a) Facial weakness and ptosis
b) Sensory Loss
c) Signs of autonomic disturbances Eg: Postural Hypotension
a. Pain and numbness in hand and feet
d) Absent or reduced reflexes
e) Ataxia and skeletal weakness

DISEASES OF PERIPHERAL NERVES

Neuropathy is a group of diseases of nerves not associated with inflammation. They are classified as.

I. Parenchymal (polyneuropathy) -several neurons are affected.

II. Interstitial (mononeuropathy) -A single neurone is usually affected

I. Parenchymal (polyneuropathy)

Damage to a number of neurons and their myelin sheaths (ie. The long neurons are usually affected first**.** Those supplying the feet and legs.

II. Interstitial neuropathy (mononeuropathy)

Single nerve involved and the most common causes due to pressure.

a).compression: compression of radial nerve against humerus.

b).Entrapment:Carpal Tunel Syndrome-compression of median nerve as it passes through the carpal tunel in the flexor retinaculam at the wrist.

ANIMAL MODELS OF NEUROPATHIC PAIN

1. **Chemical Induced Model**

a) Acrylamide induced neuropathy.

2. Disease Induced model

a) Diabetes induced model.

b) Cancer pain model.

3. Peripheral nerve injury model

a) Chronic constriction injury model

b) Complete sciatic nerve transition model

c) Partial sciatic nerve ligation model

d) Spinal nerve ligation model

e) Tibial and sural nerve transition model

f) Common peroneal nerve ligation model

4. Drug induced model

a) Vincristine induced model

b) Anti HIV drug induced model

5. Micellaneous model

a) Chronic ethanol consumption/withdrawal induced neuropathy

b) Pyridoxine defficiency induced neuropathy

NEUROPATHY CURES BY HERBS

Neuropathy is a term that refers to problems associated with the peripheral nervous system and it may cause muscle weakness, spasms, cramps, and pain. These symptoms can be easily triggered by outside stimuli and they may occur over again unless treated. There are some herbal remedies that can cure or treat these conditions without the side effects of allopathic medicine.

- *Hypericum perforatum*
- *Polygonum multiflorum*.
- *Levisticum officinale*
- *Filipendula ulmaria*.

HEPATOCELLULAR CARCINOMA (HCC)

This is the most common form of liver cancer in adults. It begins in the hepatocytes, the main type of liver cell. About 3 out of 4 cancers that start in the liver are this type. HCC may have different growth patterns.[6]

- Some start as a single tumor that grows larger. Only late in the disease does it spread to other parts of the liver.
- Others seem to start in many spots throughout the liver, not as a single tumor. This is most often seen in people with ongoing liver damage (cirrhosis) and is the most common pattern seen in the United States.

Pathologist can figure out the subtypes of hepatocellular cancer by looking at the cancer under a microscope. Most of these subtypes do not affect treatment or the patient's outlook. But one rare type, called *fibrolamellar,* has a much better outlook (prognosis) than other forms of liver cancer.

An estimated 26,190 new cases of liver cancer (including intrahepatic bile duct cancers) are expected to occur in the US during 2011. More than 80% of these cases are hepatocellular carcinoma (HCC), originating from hepatocytes, the predominant type of cell in the liver. The incidence of liver cancer has been increasing by 3.4% per year in men and by 3.0% per year in women since 1992. In contrast to most common cancer sites, incidence rates are highest among Asian Americans/Pacific Islanders and Hispanics.

Liver cancer (hepatocellular carcinoma) is a cancer arising from the liver. It is also known as primary liver cancer or hepatoma. The liver is made up of different cell types (for example, bile ducts, blood vessels, and fat-storing cells). However, liver cells (hepatocytes) make up 80% of the liver tissue. Thus, the majority of primary liver cancers (over 90%-95%) arises from liver cells and is called hepatocellular cancer or carcinoma.

SCOPE OF THE LIVER CANCER PROBLEM

Liver cancer is the third most common cancer in the world. A deadly cancer, liver cancer will kill almost all patients who have it within a year. In 2000, it was estimated that there were about 564,000 new cases of liver cancer worldwide, and a similar number of patients died as a result of this disease. About three-quarters of the

cases of liver cancer are found in Southeast Asia (China, Hong Kong, Taiwan, Korea, and Japan).

The frequency of liver cancer in Southeast Asia and sub-Saharan Africa is greater than 100 cases per 100,000 populations.

Cancer a dreadful disease occurring due to anomalybin mitotic cell cycle is the second cause of death in developed countries, approximately one in five die of cancer, According to WHO out of an estimated total of 50 million deaths annually in the world more than five million are attributed to cancer and the number of deaths from throughout the world is increasing approximately 500,000 new case of cancer are observed. Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and its incidence in the United States and other countries has been steadily increasing over the past 25 years.

Causes, incidence, and risk factors

Hepatocellular carcinoma accounts for most liver cancers. This type of cancer occurs more often in men than women. It is usually seen in people age fifty or older. However the age varies in different parts of the world. The disease is more common in parts of Africa and Asia than in North or South America and Europe. Hepatocellularcarcinomais not the same as metastatic liver cancer, which starts in another organ (such as the breast or colon) and spreads to the liver. In most cases, the cause of liver cancer is usually scarring of the liver (cirrhosis). Cirrhosis may be caused by:

- Alcohol abuse (the most common cause in the United States)
- Autoimmune diseases of the liver

- Hepatitis B or C virus infection
- Inflammation of the liver
- Iron overload in the body (hemochromatosis)

Patients with hepatitis B or C are at risk for liver cancer, even if they have not developed cirrhosis.

Symptoms

1. Abdominal pain or tenderness, especially in the upper-right part
2. Easy bruising or bleeding
3. Enlarged abdomen
4. Yellow skin or eyes (jaundice)

SIGNS AND TESTS

Physical examination may show an enlarged, tender liver.

Tests include:

- Abdominal CT scan
- Abdominal ultrasound
- Liver biopsy
- Liver enzymes (liver function tests)
- Liver MRI
- Serum alpha fetoprotein

Some high-risk patients may get regular blood tests and ultrasounds to see whether tumors are developing.

ANTIOXIDANT APPROACH OF HERBAL MEDICINE

Herbal medicine is prepared from a variety of plant materials-leaves, stems, roots, bark and so on. Living plants may be considered as a biosynthetic laboratory not only for primary metabolites, and also secondary metabolites such as glycosides, alkaloids, flavonoids, terpenoids and tannins etc that are responsible for treating mild and chronic ailments.[8, 9,10.]

Diseases are due to the shift in the balance of pro-oxidant and antioxidant. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of the current life (or) If not effectively scanned by cellular constituents and depletion of the dietary antioxidant may leads to various disease conditions, such as rheumatoid arthritis, hemorrhagic shock, CVS disorders, neurodegenerative disease, AIDS and Gastrointestinal ulcerogenesis have been reported.

- Antioxidants are capable of protecting against the damage induced by free radicals also has a role in reducing the effects of diabetes.
- Plant materials have been used in the treatment of malignant diseases. It has been estimated that over 60% of anticancer drugs in current use are derived from plants.
- Nutrional factors and oxidative damage which plays critical role in the pathophysiology of numerous neurodegenerative diseases such as Alzheimer's and Parkinson's disease.
- Thus the evaluation of antioxidant potential of centrally acting drugs are Becoming more relevant and essential.

CHAPTER-II

REVIEW OF LITERATURE

The literature survey of *Ipomoea sepiaria Koen ex Roxb* Reveals that the following works have been already done on the plant.

ETHANOMEDICAL INFORMATION:

- The plant is extensively used as fodder for cattle.
- *Ipomoea sepiaria* plant juice have been used in folk medicine for the treatment of deobstruent oedema, diabetic mellitus and nervous disorder.
- The plant is used for sterility in women, urinary retention, constipation and gynecological disorders.
- Ipomoea stans and ipomoea batatas have been reported for anticancer activity.

Ipomoea sepiaria:

1. Syed shayfur Rahman studied the Investigation of Sapindus mukorossi extracts for repellency, Insectidal Activity and plant growth regulatory effect.[54]

2. Ray.B,et.al studied the antimicrobial activity potentiality of Ipomoea sepiaria (convolvulaceae).[51]

3. Vijayan Mini N, et.al studied the antimicrobial activity of ten common herbs,commonly known as Dashapushpam from kerala,India.[52]

4. Abdul Latif L, et.al reported the efficacy of some organic modification for the control of ufra disease of Rice.[53]

OTHER SPECIES:

- **Shukla G and Verma BK (1990)** have described the taxonomy of *Ipomoea batatas, Ipomoea carica, Ipomoea hederifolia, Ipomoea aquatia, Ipomoea pes-tigridis, Ipomoea turbinata, Ipomoea carnea, Ipomoea obscura, Ipomoea sepiaria* and *Iopomea sinensis* .[11]
- **Singh V and Gupta PC (1995)** have isolated a water soluble seed gum polysaccharide containing D-galactose and D-mannose as sugars from *Ipomoea pes-tigridis* and its structure elucidation has been described.[12]
- **Parveen F and Bhandari MM (1982)** have discussed the polynogical account of 7 genera and 23 species of Convolvulaceae found in Indian desert and pollen morphology in relation to taxonomy of the family.[15]
- **Karatela YY and Gill LS (1985)** have described the epidermal morphology and stomatal ontogency of ten plant (Convolvulaceae) species. The leaves of the plants shows amphistomatic, paracytic stomata type with eumesogenous ontogeny in all the Convolvulaceous species except in two *Ipomea* species.[16]
- **Siddiqi TO *et al.*, (1990)** have identified the botanical character of Kaladana (*Ipomoea hederarea*).[17]
- **Goto T *et al.*, (1981)** have reported the structure of an alkali hydrolysis product of heavenly blue anthocyanin (HBA) which was isolated from blue flowers of morning glory *(Ipomoea spp),and it was determined to be trans-o–[6-o-(-trans-3-o*-β-D—caffeyl)-β-D- glucopyronosyl] caffeic acid by spectral studies.[19]

- **Takagi S *et al.,* (1981)** have isolated the isoquercitrin monoacetate, isoquercitrin hyperin and quercetin sodium salt from *Ipomoea biloba* by column chromatography and were identified by thin layer chromatography.[20]
- **Dubey P *et al.,* (1982)** have described the isolation of a new flavanoid from ethyl acetate extract of *Ipomoea fistulosa* and its structure was elucidated by spectral studies.[21]
- **Schneider JA et al., (1984)** have isolated nine new sesquiterpenes from the root of *Ipomoea batatas* and their structures were also determined.[22]
- ***Harrison DA and Kulshreshtha DK (1986)*** have examined the four new fatty acid glycosides from *Ipomoea dichroa*. They were identified as dichrosides A, B, C and D besides friedelin, stearic acid, β-sitosterol and its glucosides.[23]
- **Wilkinson RE *et al.,* (1987)** have isolated the seed alkaloids from *Ipomoea hederifolia, Ipomoea coccinea and, Ipomoea wrightii* and these alkaloids were quantified by spectrophotometry as ergonovine maleate equivalents.[24]
- **Ono M *et al.,* (1991)** have isolated the ether soluble resin glycosides, operculins from the roots of *Ipomoea operculata.* These were characterized on the basis of chemical and spectral analysis.[25]
- **Ysrael MC and Waterman PG (1994)** have evaluated the isolation of antifungal and cytotoxic principles from *Ipomoea muricata*.[26]
- **Yahara S *et al.,* (2002)** have isolated the phenolic compounds from the methanolic extract of dried leaves of *Ipomoea batatas* and these compounds were identified by spectral studies.[27]

- **Kappor A *et al.*, (1981)** have demonstrated the petal extracts of *Ipomoea carnea, Ipomoea palmate* exhibit antifungal activity against *Alternarria brassicae, Alternaria brassicicola* and *Fusarium oxysporium* due to presence of some flavonoids.[30]
- **Khare AK *et al.*, (1982)** have evaluated the anti-inflammatory activity of *Ipomoea turpethinum*. The aqueous extract of root showed more potent activity (38.3) against acute, subacute and chronic inflammation in carageenan induced paw edema model compared to alcoholic (32%) and ethereal extract (26%).[31]
- **Mujumdar AM *et al.*, (1983)** have demonstrated that the ethanol extracts of *Ipomoea leari* seeds produced depression at low doses and stimulation at higher doses, ultimately which lead to convulsions in mice and at higher doses analgesic activity was noted. The antibacterial activity was also studied.[32]
- **Da silvo filho AA *et al.*, (1986)** have described the isolation of four new antimicrobial glycosides from *Ipomoea bahensis*. They showed significant activity against sarcoma 180 in mice.[33]
- **Pongprayoon U (1991)** has studied the effect of extract of *Ipomoea pes-caprae* in the treatment of dermatitis caused by poisonous jelly fish toxins. It also exhibited significant antispasmodic activity in isolated guinea pig ileum and anti-inflammatory in carageenan induced in rat paw edema.[34]
- **Reza MS *et al.*, (1994)** have studied the antibacterial activity of the chloroform extract of different parts of *Ipomoea fistulosa* against all the strains of *Shigella* and the gram positive bacteria namely *Bacillus megaterium* and *Bacillus polymyxa*. The extract exhibited significant activity.[35]

- **Reynolds WF *et al.,* (1995)** have isolated and characterized the cytotoxic and antibacterial tetrasaccharide glycoside from *Ipomoea stans*. These compounds have pronounced cytotoxicity towards three human tumor cell lines as well as specific antibiotic activity against two bacterial strains.[36]
- **Navarro-Ruiz A *et al.,* (1996)** have demonstrated the anti-convulsant effect of aqueous, hydroalcoholic and chloroform extracts from roots of *Ipomoea stans*. Maximal electroshock seizure inducing test (MES) and subcutaneously injected metrazole (METSC) were the *premental epilepsy* model used and maximum activity was seen with aqueous extract.[37]
- **Matsui T *et al.,* (2002)** have performed anti-hyperglycemic effect of diacylated anthocyanin derived from *Ipomoea batatas*. They suggested that anthocyanin had alpha glucosidase inhibitory activity after a single oral administration in 8week old male rats.[38]
- **Barnes CC *et al.,* (2003)** have reported that the organic soluble extract from the leaves of *Ipomoea leptophylla* showed activity against *M. tuberculosis (Invitro method*).[39]
- **Dhembare AJ and Sangle S (2003)** evaluated the effect of various plant extracts (*Piper nigrum, Ipomoea fistulosa*) for their antimicrobial activity against human pathogenic bacterial strains.[40]
- **Haueza IM *et al.,* (2003)** have reported the immunomodulatory activity of *Ipomoea carnea* on peritoneal cells of rat model. The experimental model suggested that low doses of *Ipomoea carnea* induced enhanced phagocytosis activity and hydrogen peroxide production by macrophages.[41]

- **Yoshimoto M *et al.*, (2004)** have estimated the phenolic composition and radical scavenging activity of sweet potato *Ipomoea batatas* treated with Koji [*Aspergillus awamori* and cellulase (cellulosin T2)].[42]
- **Prasad KN *et al.*, (2006)** have studied callus induction from *Ipomoea aquatica* Forsk leaf and its antioxidant activity. The antioxidant activity was analysed by DPPH, TBARS and metal chelating methods. Hyper antioxidant activity was observed in one month old callus produced by NAA in combination with Kinetin.[43]
- **Ferreira AA *et al.*, (2006)** have evaluated the anti-nociceptive effect from *Ipomoea cairi*ca. The ethanolic extract (100,300,1000 and 3000mg/kg; per oral) of *Ipomoea cairica* induced dose dependent reduction of response in the formalin test inflammatory phase in mice model. The same dose range did not modify neurogenic pain in formalin test, tail flick reflex latency, and carageenan induced paw edema and rota-rod test motor performed.[44]
- **Datta Choudry M *et al.*, (2007)** have studied the chemical characterization and antifungal, CNS depressant activity of *Ipomoea aquatica*. The methanolic extract of *Ipomoea aquatica* showed the antifungal activity against *Candida albicans* (1.6cm) and for CNS depressant activity in mice was as potent as standard.[45]
- **Sokeng SD *et al.*, (2007)** have demonstrated the inhibitory effect of *Ipomoea aquatica* extracts on glucose absorption using a perfused rat intestinal preparation model. The aqueous/dichloromethane/methanol extracts showed the significant inhibitory effect on glucose absorption in test animals. The most pronounced

effect was observed with the aqueous extract. Quabain was used as a reference inhibitor.[46]

- **Hamsa TB and Kuttan G (2007)** have reported the antioxidant activity of methanolic extract of *Ipomoea obscura in vitro and in vivo* models. *In vitro* model, the extract was found to have potential antioxidant activity as it could inhibit lipid peroxidation, and scavenge hydroxyl, superoxide and nitric oxide radicals generated. *In vivo* model, the extract (10mg/kg]) was found to inhibit the PMA induced superoxide generation in mice peritoneal macrophages.[47]
- **Vimala Y *et al.*, (2007)** have demonstrated the antimicrobial activity of *Ipomoea kentrochulos* on microorganisms and microbial isolates on spoiled vegetable by disc diffusion method. The minimal inhibitory effect of crude residual extract was determined by *tube dilution* against different microbes which range more than 800µg/ml.[48]
- **Rivera IL *et al.*, (2008)** have studied the anti-mycobacterial and, cytotoxicity and effects on the central nervous system of *Ipomoea tyrianthina*.[49]
- **Chimkode R *et al.*, (2009)** have evaluated the wound healing activity of tuberous root extracts of *Ipomoea batatas in vivo model*. The petroleum ether extract showed more potent wound healing activity (dead space granulation) models compared to other extracts.[50]

CHAPTER-III

AIM AND SCOPE

Ipomoea sepiaria Koen.ex Roxb. (convulvulaceae) is a glabrous or occasionally pubescent, hirsute,slender twiner with a slightly thickend or tuberous perennial root and short stem producing annual or seasonal,It is available throughout india in the plains especially near the coast and up to an elevation of about 500 feet in the hills,It also grows near water margin. This plant is used in traditional medicine as a popular remedy to treat various ailments.

The ethanomedical information revealed that almost all parts of this plant are employed in various indigenous system of medicine against several diseases.

- The plant is extensively eaten as pot herb also used as fodder for cattle.
- *Ipomoea sepiaria* plant juice has been used in folk medicine for the treatment of deobstruent, diuretic, diabetic and nervous disorders.
- This plant is also used for sterility in women, urinary retention, constipation and gynecological disorders.
- Ipomoea batatas and ipomea stans have been reported for anti cancer activity.

According to the information available from the literature survey, this plant has not been reported for painful neuropathy though ethanomedical information indicates that it has been used for the treatment of nervous disorders. Pharmacognostical and Phytochemical studies also not been reported for the leaves of this plant.

So, the present work has been designed to carry out the following works on the leaves and stems of *Ipomoea sepiaria* .

1. It is planned to carry out the detailed Pharmacognostical studies on the leaves and stem of this plant.
2. Preliminary phytochemical studies were carried out for the crude drug as well as extracts, in order to confirm the presence of primary and secondary metabolites like proteins, carbohydrates, terpenoids, flavonoids and tannins.
3. To estimate the total phenolic, and flavonoid content in the extracts of this plant.
4. Phytochemical evaluation including isolation, identification and characterization of secondary metabolites.
5. It is planned to evaluate the extract of this plant for the **following pharmacological studies.**

a. Determination of antioxidant potential of extracts by,

i Hydrogen peroxide scavenging activity.

b. Evaluation of antioxidant potential of extracts and isolated compounds by,

ii DPPH (2,2,diphenyl-2-picryl hydrazyl) assay.

c. Pharmacological Evaluation of *Ipomoea sepiaria* on Acrylamide induced neuropathic pain in rats.

d. Pharmacological Evaluation of *Ipomoea sepiaria against* invitro anti cancer activity for HepG2 cancer cell lines.

CHAPTER-IV

PHARMACOGNOSTICAL STUDIES

Ipomoea sepiaria .Koen. ex Roxb. is a slender twinning perennial plant belonging to the family convolvulaceae.

SECTION-A

SYSTEMATIC POSITION

Kingdom	**:**	Plantae
Subkingdom	**:**	Tracheobionta
Division	**:**	Magnoliophyta
Super division	**:**	spermatophyta
Class	**:**	Magnoliopsida
Subclass	**:**	Asteridae
Order	**:**	Solanales
Family	**:**	convolvulaceae
Genus	**:**	*Ipomea*
Species	**:**	*sepiaria*

VERNACULAR NAMES

English	:	Ipomoea sepiaria
Tamil	:	Talikkirai, manjigai,
Telugu	:	Mettatuti, puritititige
Hindi & Bengali	:	Bankalmi
Malayalam	:	Tirutali
Oriya	:	Bilona, Mushakani
Marathi	:	Amtivel
Gujarathi	:	Hanumanvels

GEOGRAPHICAL DISTRIBUTION

Herbaceous slender, perennial twinner found almost throughout india in hedges,near streams and tanks.

HABIT AND HABITAT OF PLANT

Ipomoea sepiaria is a glabrous or occasionally pubescent or hirsute, slender producing annually or seasonally a number of terete villous, greyish purple branches bearing simple cordate or ovate-cordate,variable medium size leaves blotched with brownish or purplish patches towards the centre.

DESCRIPTION OF THE PLANT:

The plant is a slender vine. It is found in the plains and on thickness of dry soil. The leaves are triangular-cordiform, glabros, base cordate, apex acute, petiole up to 2cm long flowers are umbellate cymes. Calyx lobes are unequal corolla cream colour with purple throat. Stamens are fine and unequal, Ovary conical in shape stigma capitate, fruit are capsule and the seeds are white velvety.[55]

FOLIAGE

Leaf arrangement	:	simple alternate
Leaf type	:	petiolate
Leaflet margin	:	entire
Leaflet shape	:	ovate-cordate
Leaflet venation	:	less dense and reticulate
Leaf color	:	dark green
Size	:	1.5 inch in long

FLOWER

Flower color	:	Pale purple, pink or white
Flower characteristics	:	Pedicelled, sepals five ovate or broadly elliptic, shortly apiculate, glaborous with membraneous margin.

FRUIT

Fruit shape	:	Ovoid capsule
Fruit length	:	One fourth to one third inch in diameter
Fruit covering	:	Minutely tawny and velvety
Fruit color	:	Grey

SEED

Light grey seeds usually 4 or 6 glaborous velvety or woolly 0.15 inch.

SECTION-B

MICROSCOPICAL STUDY OF THE LEAVES

MATERIALS AND METHODS

Leaves and stem of *Ipomoea sepiaria* was collected from road side of Madurai. Care was taken to select healthy plants and normal organs. Samples of leaf and stem were cut and removed from this plant and fixed in FAA (formalin-5ml+acetic acid-5ml+70%ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the standard procedure

Infiltration of the specimens were carried out by gradual addition of paraffin wax (melting point 58-60 c) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.[56, 57, 58, 59, 60 , 61]

SECTIONING

The paraffin embedded specimen was sectioned with the help of rotary microtome. The thickness of the sections was 10-12µm, dewaxing of the sections was by customary procedure.. The sections were stained with toludine blue as per the method published by O'BRIEN et al. (1964). Since toludine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with saffranin and fast-green and iodine (for starch) For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of

leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass,1940) were prepared. Glycerin mounted temporary preparations were made for macerated/ cleared material. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin after staining. Different cell component were studied and measured.

PHOTOMICROGRAPHS

Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnification of the figures was indicated by the scalebars.

Anatomical features:

Leaf:

The leaf consists of fairly prominent midrib and thick smooth lamina. The midrib consists of a broad short adaxial conical hump and wide convex abaxial part (fig 5.1). The midrib is 580µm thick and the adaxial cone is 250µm wide. The conical part includes a small mass of collenchymas and the remaining portion of the midrib consists of thin walled compact parenchymatous ground tissue. The palisade tissue extends up to the lateral part of the collenchyma mass. The vascular strand in single and fan-shaped in sectional view. It is 150µm thick and 250µm wide. The vascular strand consists of parallel rows of thin walled angular xylem elements and small groups of phloem elements both on the lower and upper ends of the vascular bundle (fig.5.1).

Lateral view:

The lateral vein is slightly thicker than the lamina; it is 450µm thick. It consists of a single circular vascular strand surrounded by parenchymatous ground tissue. The vascular bundle comprises cluster of small angular xylem elements and a thin arc of phloem elements placed on the lower end. The vascular bundle is 150µm wide.

LAMINA (fig.5.3):

The lamina is 350µm thick. It is dorsiventral with distinct adaxial zone of palisade tissue and abaxial spongy parenchyma. The adaxial epidermis consists of fairly thick rectangular thin walled epidermal cells; the abaxial epidermis is comparatively thin, the cells being narrowly cylindrical. Both adaxial and abaxial epidermal layers are stomataferous. The palisade layer is single layered and the cells are short, wide and cylindrical. The spongy parenchyma cells are four or five layered cells being lobed and loosely arranged.

EPIDERMAL CELLS AND STOMATAL MORPHOLOGY:

The epidermal tissue was studied from the paradermal sections.The epidermal cells are angular and thick walled. The stomata are exclusively paracytic type.Each stoma has two or occasionally three subsidiary cells lying parallel to the guard cells. The stomata are elliptical in shape measuring 20x30µm in size.

VENATION OF THE LAMINA (fig.7.1,2):

The venation pattern is less dense reticulate; the shape and size of the vein-islets. The veins are thin and undulate. The vein terminations are long, slender, curved and simple. Most of the terminations bud in a cluster of terminal-tracheids (fig.7.2). The tracheids are rectangular and square shaped with spiral thickenings.

PETIOLE (fig.8)

The petiole is circular in sectional view with adaxial wide V-shaped groove. It is 1.2mm thick and 1.1mm wide. The epidermal layers of the petiole consists of fairly wide, thin walled rectangular or squarish cells with thin cuticle. Two or three outer layers of ground tissue are collenchymatous, the remaining portions being parenchymatous. The vascular system consists of a shallow arc of larger main bundle and two smaller circular wing bundles. The main bundle includes several short parallel rows of xylem elements and small groups of phloem units distributed both along the adaxial and abaxial sides of the xylem.

POWDER MICROSCOPIC OBSERVATIONS:

EPIDERMAL GLANDULAR TRICHOMES:(fig.9.1,2,3)

Glandular trichomes are common on both adaxial and abaxial sides of the lamina. The adaxial epidermal cells are polygonal with thick straight anti clinal walls. The gland is a circular plate comprising about 8 triangular cells bounded by a thin cuticular membrane. The gland is 70µm in diameter (fig.9.1)

The glandular trichomes on the abaxial side are similar to those on the adaxial side,except that the glands are smaller and thin walled (fig.9.3). The gland is 50µm in diameter.

EPIDERMAL TISSUE (fig.10.1,2. 11.1,2):

Fragments of epidermal peelings are frequently seen in the powder. They are seen in surface view. The abaxial epidermis tissue differ from the adaxial epidermis in shape and size of the cells as well as in the frequency of the stomata (compare fig.10.1 and 11.1). The abaxial epidermal cells are larger in size their anticlinal walls are thin and wavy; the cells are amoeboid in outline. The stomata are paracytic with more or less equal subsidiary cells (fig.10.2). The adaxial epidermal cells are smaller in size. Their anticlinal walls are thick and straight (fig 11.1). The stomata are paracytic type

and the subsidiary cells are mostly unequal in size (fig.11.2). The surface of the cells appears finely dotted cuticular markings.

MICROSCOPIC FEATURES OF THE STEM:

The stem is circular with even surface, frequently seen on certain places (fig-12.1,2). The stem is 1.2mm in thick. The epidermis is radially oblong , thin walled and wide. The cells are 20mm thick. Linear to the epidermis is seen the initial stage of periderm formation, which includes 2 or 3 layers of phloem.

THE CORTEX:

The cortical cells are crushed in to dark layers of rectangular cells. The cortical zone is formed by a wide distinct zone of outer secondary phloem (fig-12,3). The outer phloem elements are wide, polygonal and are random in arrangement. It includes sieve elements and phloem parenchyma cells.

SECONDARY XYLEM:

The xylem cylinder is somewhat elliptical in outline. Two opposite ends are thicker with inner primary xylem and outer secondary xylem. The other opposite ends are thin and possess primary xylem and lacks the secondary xylem (fig.12,2). The primary xylem consists of two several compact rows of cells, with two or three xylem elements in each row. Secondary xylem is at the initial stage if development. It includes solitary wide, circular vessels and thick walled fibers.

The vessels are 130µm wide (fig.12.3) along the inner zone of the primary xylem occurs the inner phloem (also called medullary phloem or interxylary phloem). The inner phloem consists of isolated masses of phloem elements and parenchyma cells (fig.12.4).

Calcium oxalate druses are common in the pith cells. The druses solitary and occurs in unmodified parenchyma cells, they are solitary and 20µm thick (fig.12.5).

SECTION- D

QUANTITATIVE MICROSCOPY

DETERMINATION OF LEAF CONSTANT:

The vein islet number, vein termination number, stomatal number and stomatal index were determined on fresh leaves using standard procedure.

A. Vein islet number and vein termination number:

The term vein islet is used to denote the minute area of photo synthetic tissue encircled by the ultimate division of the conducting strands. The number of vein islets per square mm area is called vein- islet number.

Vein termination number may be defined as the number of vein terminations present in one square mm area of the photosynthetic tissue.

Determination of vein islet number and vein termination number:

Pieces of leaves were cut from various regions of the leaves between midrib and the margin, cleared in chloral hydrate and mounted on a slide. Camera Lucida and drawing board were arranged. With the help of a stage micrometer, camera Lucida and microscope, 1mm square was drawn on the paper. Then the stage micrometer was replaced by the preparation and the veins were traced in that square. Then the vein islets and vein terminations were counted in the square. Ten such readings were taken and the average was calculated and the results were presented in **TABLE-I.**

TABLE -I

VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER OF THE LEAVES OF *Ipomoea sepiaria*

OBSERVATION NUMBER	VEIN ISLET NUMBER	VEIN TERMINATION NUMBER
1	7	8
2	8	6
3	8	7
4	9	9
5	7	9
6	7	6
7	10	8
8	8	9
9	7	7
10	7	7

RANGE	MINIMUM	AVERAGE	MAXIMUM
Vein islet	7	8	10
Vein termination	6	8	9

A. Stomatal Number:

The average number of stomata per square mm area of epidermis of the leaf is called stomatal number.

Determination of Stomatal Number:

Pieces of upper and lower epidermal peelings were mounted on a slide With the help of camera Lucida and stage micrometer 1 mm square was drawn on a paper.

The stage micrometer was replaced by the preparation. Then the preparation was observed under high power and the stomata marked in that unit area. Number of stomata present in those unit areas was calculated. Ten such readings were taken and the average of stomatal number was calculated and presented in the TABLE-II.

TABLE- II

STOMATAL NUMBER OF THE LEAVES OF *Ipomea sepiaria*

OBSERVATION NUMBER	**LOWER EPIDERMIS**
1	29
2	32
3	27
4	24
5	30
6	34
7	28
8	31
9	27
10	23

RANGE	**MINIMUM**	**AVERAGE**	**MAXIMUM**
Lower epidermis	23	29	34

A. Stomatal Index:

Definition:

It is the percentage which the numbers of stomata form to the total number of epidermal cells, each stoma being counted as one cell.

Stomatal index $S.I = \frac{S}{E+S} \times 100$

Where, S=number of stomata per unit area

E= number of epidermal cells in the same unit area

Determination of Stomatal Index:

The procedure adopted in the determination of stomatal number was followed and then the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula and the results were given in **TABLE-III**.

TABLE - III

STOMATAL INDEX OF THE LEAVES OF *Ipomoea sepiaria*

OBSERVATION NUMBER	LOWER EPIDERMIS
1	11.11
2	8.57
3	8.57
4	13.51
5	17.95
6	8.57
7	13.51
8	11.11
9	5.88
10	15.79

RANGE	MINIMUM	AVERAGE	MAXIMUM
LOWER EPIDERMIS	5.88	11.46	17.95

SECTION-E

PHYSICAL PARAMETERS

The Physical parameters such as Total ash, Acid insoluble ash, Water soluble ash, Extractive values and Loss on drying were determined separately for air dried powdered leaves of this plant as per the official method.

A.ASH VALUES:

The ash values were determined by using air dried powdered leaves as per the official method.

i. Total ash:

2 grams of the crude leaf powder was accurately weighed in a tarred nickel crucible. The ground drug was scattered in a fine even layer on the bottom of the crucible and incinerated by gradually increasing the heat not exceeding 450˚c [dull red heat] until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried drug was calculated .

ii. Acid insoluble ash:

The ash obtained from the total ash was boiled for 5 minutes with 25ml of 2M hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, ignited to constant weight, cooled in a desiccator and weighed. The percentage of acid insoluble ash with reference to the air – dried drug was calculated.

iii. Water soluble ash:

For water soluble ash, ash obtained from the total ash was boiled with 25ml of distilled water. The insoluble matter was collected in a Gooch crucible, washed with hot water ignited to a constant weight. Cooled in a desiccator and weighed. The weight of the insoluble matter was subtracted from the weight of the ash. The differences gave the weight of the water soluble ash. It was calculated with reference to the air-dried powder. The results were represented in **TABLE-IV.**

TABLE- IV

ASH VALUES

OBSERVATION NUMBER	**TOTAL ASH %W/W**	**ACID INSOLUBLE ASH %W/W**	**WATER SOLUBLE ASH %W/W**
1	16.31	6.82	-
2	15.18	6.55	-
3	15.63	5.97	-
4	14.92	6.45	-
5	16.88	5.65	-
6	16.09	-	10.06
7	15.34	-	9.83
8	15.47	-	9.66
9	15.24	-	10.14
10	15.87	-	10.02
MINIMUM	**14.92**	**5.65**	**9.83**
MAXIMUM	**16.88**	**6.82**	**10.14**
AVERAGE	**15.69**	**6.55**	**9.89**

B. Determination of Extractive values

i. Alcohol (Ethanol) soluble extractive:

5 gm of the air –dried drug, coarsely powdered, was macerated in 100ml of ethanol in a closed flask for 24 hours, shaking frequently during 6 hrs and allowed to stand for 18 hrs, filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚ c, to constant weight. The percentage of alcohol soluble extractive with references to the air - dried drug was calculated and presented in **TABLE-V.**

ii. Determination of Water-Soluble Extractive:

5 gm of the air dried drug coarsely powdered, was macerated in 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during 6 hours, and allowed to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred-flat-bottomed shallow dish and dried at 105˚ C to constant weight. The percentage of water – soluble extractive with reference to the air-dried drug was calculated and presented in **TABLE –V.**

iii. Determination of Petroleum Ether soluble Extractive:

5 gm of the air –dried drug, coarsely powdered, was macerated in 100ml of ethanol in a closed flask for 24 hours, shaking frequently during 6 hrs and allowed stand for 18 hrs. Thereafter it was filtered rapidly, taking precautions against loss of

petroleum ether. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚ c, to constant weight. The percentage of petroleum ether soluble extractive with references to the air - dried drug was calculated and presented in **TABLE-V.**

iv. Determination of Chloroform, Methanol, Ethyl acetate and Acetone soluble extractives:

The procedure followed for the determination of alcohol soluble extractive value was adopted for the determination of benzene soluble extractive, Chloroform soluble extractive, methanol soluble extractive, ethyl acetate soluble extractive and acetone soluble extractive. Instead of alcohol, respective solvents were used for the determination of their extractive values.The percentage of chloroform, methanol, ethyl acetate and acetone soluble extractives with reference to air dried drug was calculated and presented in **TABLE-V.**

TABLE-V.

TOTAL EXTRACTIVE VALUES:

S.NO	SOLVENT	EXTRACTIVE VALUE %
1	Petroleum ether	4.95
2	Chloroform	7.68
3	Ethyl acetate	8.12
4	Acetone	10.80
5	Methanol	16.61
6	Ethanol	7.12
7	70% Ethanol	19.36
8	Aqueous	10.76

C. Loss on drying:

For the determination of loss on drying, the method described by Wallis was followed. 2 grams of the powdered crude drug was accurately weighted in a tarred dish and dried in an oven at 100˚ C. It was cooled in a desicator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken and presented in **TABLE –VI.**

TABLE -VI

LOSS ON DRYING

OBSERVATION NUMBER	**LOSS ON DRYING % W/W**		
1	11.37		
2	10.63		
3	10.03		
4	9.92		
5	10.71		
RANGE	**MINIMUM**	**AVERAGE**	**MAXIMUM**
Leaves &Stem powder	**9.92**	**10.53**	**11.37**

CHAPTER –V

PHYTOCHEMICAL STUDIES

The leaves and stem of *Ipomoea sepiaria* were collected in Madurai Dist, Tamilnadu and authenticated by Taxonomist.The shade dried leaves were powdered and then subjected to the following preliminary phytochemical tests.[66-70.]

SECTION-A

ORGANOLEPTIC EVALUATION

1.	**Nature**	-	Coarse powder
2.	**Colour**	-	Green in colour
3.	**Odour**	-	Characteristic odour
4.	**Taste**	-	Mild astringent

Powdered plant material and extracts were subjected to the following chemical tests and the results were presented in the table No **VII.**

SECTION-B

B. QUALITATIVE CHEMICAL EXAMINATION OF THE POWDER OF

Ipomoea sepiaria

1. TEST FOR STEROLS

The powdered leaf was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

a. Salkowski's Test

A few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in color indicating the presence of sterols.

.b. Liebermann – Burchard's Test

To the chloroform solution a few drops of acetic anhydride and 1 ml of concentrated Sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

2. Test for Terpenoids

A little of the powdered leaf was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. The colour of the solution turned to pink indicates the presence of terpenoids.

3. TEST FOR CARBOHYDRATES

a. Molisch's Test:

The aqueous extract of the powdered leaf when treated with alcoholic solution of α-naphthol in the presence of sulphuric acid gave purple color indicating the presence of carbohydrates.

b. Fehling's Test:

The aqueous extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

4. TEST FOR FLAVONOIDS

a. Magnesium turning- con HCl test:

A little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red or magenta color was obtained indicating the presence of flavonoids.

b. Alkali Test

To the small quantity of test solution 10% aqueous sodium hydroxide Solution was added. Yellow orange color was produced indicating the presence of flavonoids.

C. Acid Test

To the small quantity of test solution, few drops of concentrated Sulphuric acid were added. Yellow to crimson color was obtained indicates the presence of flavonoids.

7. TEST FOR PROTEINS

a. Millon's Test

A small quantity of aciduous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating Indicate the presence of proteins.

b. Biuret Test

To another portion of aciduous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution was added, followed by this one drop of dilute copper sulphate solution was added. Violet color was obtained indicating the presence of proteins.

8. TEST FOR ALKALOIDS

a. About 2gm of the powdered material was mixed with 1gm of calcium hydroxide and 5ml of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200ml of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5ml of dilute hydrochloric acid was added followed by 2ml of each of the following reagents.

a) Mayer's Reagent - No cream precipitate

b) Dragendorff's Reagent - No orange brown precipitate

c) Hager's Reagent - No Yellow precipitate

d) Wagner's Reagent - No Reddish brown precipitate

b. Test for Purine group (Murexide test)

The residue obtained after the evaporation of chloroform as described in (a) was treated with 1ml of hydrochloric acid in a porcelain dish and 0.1gm of Potassium chlorate was added and evaporated to dryness on a water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple Color was obtained indicating the absence of purine group of alkaloids.

9. Test for Glycosides

a. Borntrager's Test

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate, benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly.

No color reaction was observed in ammoniacal layer showing the absence of Anthraquinone glycosides.

b. Modified Borntrager's Test

About 0.1g of the powdered drug was boiled for 2minutes with dilute Hydrochloric acid and few drops of ferric chloride solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the Benzene extract.

No pink color was observed in ammoniacal layer showing the absence of anthraquinone glycosides.

10. TEST FOR CARDIAC GLYCOSIDES (FOR DEOXYSUGAR)

a. Keller Kiliani Test

About 1g of the powdered leaf was boiled with 10ml of 70% alcohol for 2 minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of Solution of lead acetate were added and filtered, evaporated to dryness. The Residue was dissolved in 3 ml of glacial acetic acid. To this 2 drops of ferric chloride solution was added. Then 3 ml of concentrated sulphuric acid was added to the sides of the test tube carefully and observed.

No reddish brown layer was observed indicating the absence of deoxysugars of cardiac glycoside

b. Test for Cyanogenetic Glycosides

Small quantity of the powder was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place. No brick red color was produced on the paper indicating the absence of Cyanogenetic glycosides.

11. TEST FOR SAPONINS

About 0.5g of the powdered drug was boiled gently for 2 minutes with 20ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously. Frothing was produced indicating the presence of saponins.

12. TEST FOR TANNINS

A small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added.

Bluish black color was produced indicating the presence of tannins.

13. TEST FOR THE PRESENCE OF VOLATILE OIL

Weighed quantity (250 gm) of fresh leaves was subjected to hydrodistillation using volatile oil estimation apparatus (BP 1980).

No oil was collected indicating its absence in fresh leaves.

14. **TEST FOR MUCILAGE**

Few ml of aqueous extract prepared from the powdered crude drug was treated with ruthenium red. No red colour reaction indicating the absence of Mucilage.

TABLE –VII

PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE POWDER OF

Ipomoea sepiaria

S.NO	TEST	RESULTS
1.	**TEST FOR STEROLS**	
	a. Salkowski's test	+
	b. Libermann- burchard's test	+
2.	**TEST FOR CARBOHYDRATES**	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
3.	**TEST FOR PROTEINS**	
	a. Millon's test	+
	b. Biuret test	+
4.	**TEST FOR ALKALOIDS**	
	a. Mayer's reagent	-
	b. Dragendroff's reagent	-
	c. Hager's reagent	-
	d. Wagner's reagent	-
	e. Test for Purine group (Murexide test)	-
5.	**TEST FOR GLYCOSIDES**	
	a. Anthraquinone glycosides	
	i) Borntrager's test	-
	ii) Modified Borntrager's test	-
	b. Cardiac glycosides	
	i) Keller Killiani test	-
	c. Cyanogenetic glycosides	-
6.	**TEST FOR SAPONINS**	
	Foam Test	+
7.	**TEST FOR TANNINS**	
	$Fecl_3$ test	+
8.	**TEST FOR FLAVONOIDS**	
	a. Shinoda test	+
	b. Alkali test	+
	c. Acid test	+
9.	**TEST FOR TERPENOIDS**	+
10.	**TEST FOR VOLATILE OILS**	-
11.	**TEST FOR MUCILAGE**	-

(+) indicates positive reaction (-) indicates negative reaction

TABLE- VIII

PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE VARIOUS EXTRACTS OF LEAF POWDER OF *Ipomoea sepiaria*

Tests	Pet.ether Extract	Chloroform Extract	Ethyl acetate extract	Acetone Extract	Methanol Extract	Ethanol Extract	Aqueous extract
I. Test for Sterols							
a. Salkowski's test	+	+	+	+	+	+	-
b. Libermann-burchard's test	+	+	+	+	+	+	-
II. Test for Carbohydrates							
a. Molisch's test	-	-	-	-	+	+	+
b. Fehling's test	-	-	-	-	+	+	+
c. Benedict's test	-	-	-	-	+	+	+
III.Test for Proteins							
a. Millon's test	-	-	-	-	+	+	+
b. Biuret test	-	-	-	-	+	+	+
IV.Test for Alkaloids							
a.Mayer's reagent	-	-	-	-	-	-	-
b. Dragendroff's reagent	-	-	-	-	-	-	-
c. Hager's reagent	-	-	-	-	-	-	-
d. Wagner's reagent	-	-	-	-	-	-	-
e. Test for Purine group (Murexide test)	-	-	-	-	-	-	-
V.Test for Glycosides							
a. Anthraquinone glycosides	-	-	-	-	-	-	-
i) Borntrager's test	-	-	-	-	-	-	-
ii) Modified Borntrager's test	-	-	-	-	-	-	-
b. Cardiac glycosides							
i) Keller Killiani test	-	-	-	-	-	-	-
c. Cyanogenetic glycosides	-	-	-	-	-	-	-
VI.Test for Saponins	-	-	-	-	+	+	+

VII.Test for Tannins							
$Fecl_3$ test	-	-	-	+	+	+	+
VIII.Test for Flavonoids							
a. Shinoda test	-	-	-	+	+	+	+
b. Alkali test	-	-	-	+	+	+	+
c. Acid test	-	-	-	+	+	+	+
IX.Test for Terpenoids	+	+	+	+	+	+	-
X.Test for Volatile Oils	-	-	-	-	-	-	-
XI.Test for Mucilage	-	-	-	-	-	-	-

(+) Indicates positive reaction (-) Indicates negative reaction

SECTION-C

ESTIMATION OF TOTAL PHENOLIC COMPOUNDS

Principle

The total phenolic content of the *Ipomoea sepiaria* extract was determined by **Folin – Ciocalteu** Reagent method. This regeant consists of a mixture of phosphotungstate and phosphomolybdate which is reduced, during oxidation of the phenolic substances, into a mixture of blue molybdenum and tungsten oxides. The intensity of colour is proportional to the amount of oxidized phenolic compounds and it can be estimated as gallic acid equivalents at 765nm.[71, 72]

Materials Required

1. Ethanolic extracts of *Ipomoea sepiaria*
2. Gallic acid
3. 10% sodium carbonate solution
4. Folin–Ciocalteu reagent

Procedure

The total phenol content of various leaf extracts of *Ipomea sepiaria* was estimated using **Folin–Ciocalteu** reagent method. The various extracts (methanol, ethanol, acetone and aqueous) 0.1ml from 1mg/ml was transferred into separate test tubes. To this solution, Folin–Ciocalteu Reagent 0.5ml and 1ml of sodium carbonate were added and final volume was made up to 10ml with distilled water. The mixture was allowed to stand for 1 hour with intermittent shaking. The absorbance of the reaction mixture was measured at wavelength 765nm. A calibration curve was generated using absorbance reading of gallic acid at different concentrations (2, 4, 6, 8, 10µg/ml). The reaction mixture without sample was used as the blank. The total phenolic content in the various extracts were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g) and the results were presented in table no:IX

TABLE-IX

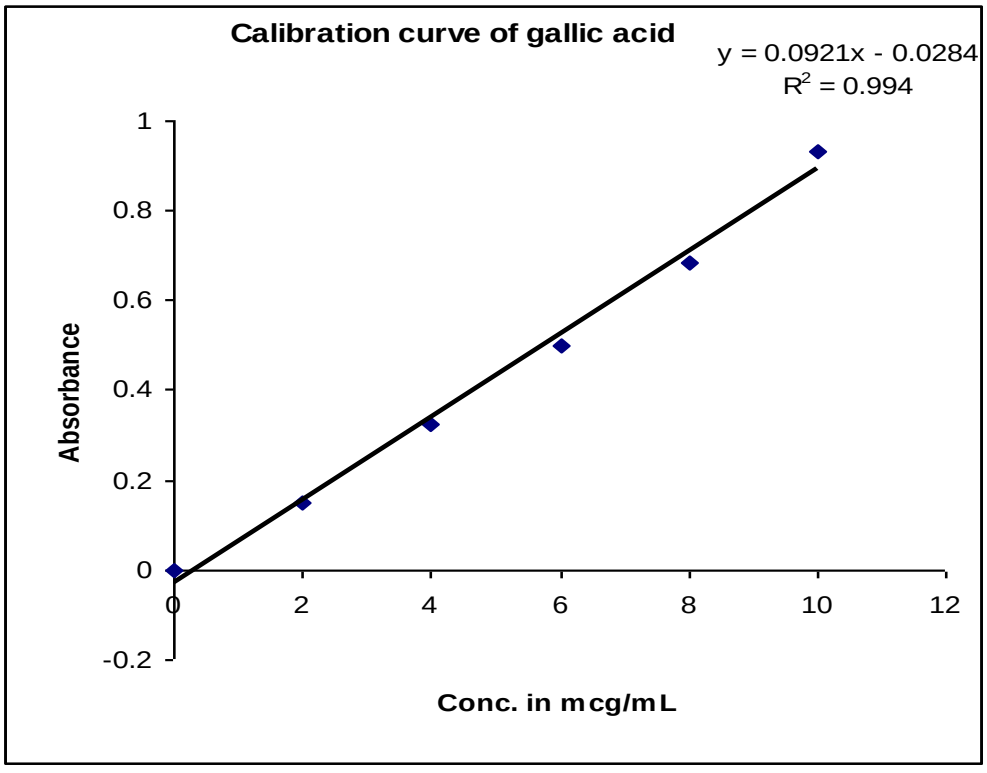
Estimation of total phenol content in ethanolic extract of

Ipomoea sepiaria.

STANDARD			TEST					TOTAL PHENOL CONTENT
	Conc. in µg/ml	Absorbance	Exracts	Conc in µg/ml	Absorbance			mg of GAE equivalent/gm of extract*
					I	II	III	
Gallic acid	2	0.148	EEIS	10	0.156	0.175	0.154	206.41±5.970
	4	0.325						
	6	0.502						
	8	0.686						
	10	0.931						

*¯mean of three readings ± SEM

Fig: 1 CALIBRATION CURVE OF GALLIC ACID



*¯mean of three readings ± SEM

ESTIMATION OF TOTAL FLAVONOID CONTENT

Principle

The total flavonoid content of various extracts were estimated by aluminum chloride colorimetric assay method. In this method, aluminum chloride complexes with standard and test sample (aluminum chloride complexes with flavonoids of C_3-C_5 hydroxyl group) and to produce intense colour in acidic medium. The intensity of the colour is proportional to the amount of flavonoids and can be estimated as quercetin equivalents at wavelength of 415 nm.[73, 77]

Materials Required

1. Ethanolic extracts of *Ipomea sepiaria*
2. 10% aluminum chloride
3. 1M potassium Acetate
4. 95% ethanol

Procedure

About 0.5ml from 1mg/ml of ethanolic extract of Ipomoea sepiaria was transferred into separate test tubes. To this solution, 0.1 ml of aluminum chloride, 0.1ml of potassium acetate and 1.5ml of 95% ethanol were added. The final volume was made up to 5ml with distilled water. The mixture was allowed to stand for 30min with intermittent shaking. The absorbance of reaction mixture was measured at 415nm. A calibration curve was generated using absorbance readings of quercetin at different concentrations (5-50µg/ml). The reaction mixture without aluminium chloride was used as the blank. The total flavonoid content in the various extracts were expressed as milligrams of quercetin equivalent per gram of extract and the results were presented in table no: 10.

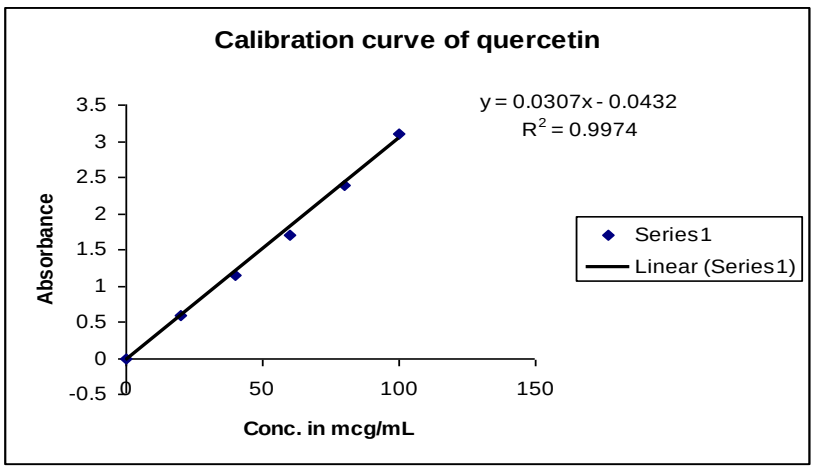
TABLE-X

ESTIMATION OF TOTAL FLAVANOID CONTENT IN ETHANOLIC EXTRACT OF *Ipomoea sepiaria*

STANDARD		**ABSORBANCE AT 415 nm**	**TEST**		**ABSORBANCE**			**TOTAL FLAVONOID CONTENT**
Sample	Conc.in µg/ml		Sample	Conc in µg/ml	I	II	III	mg of quercetin Eq/gmof extract
Quercetin	2 4 6 8 10	0.589 1.151 1.710 2.390 3.112	**EEIS**	100	0.256	0.261	0.258	194.88 ± 1.54

Mean of three readings ± SEM

Fig: 3 CALIBRATION CURVE OF QUERCETIN



SECTION –D

ISOLATION AND CHARACTERIZATION OF FLAVONOIDS. [98, 99,100]

Ethanolic extract of *Ipomoea sepiaria* was subjected to column chromatography for the isolation of phytoconstituents.

MATERIALS REQUIRED

Column

Silica gel (60 -120 mesh size)

Cotton

Petroleum ether AR grade

Ethyl acetate AR grade

Methanol AR grade

Pre-Coated TLC plates

PROCEDURE

Column chromatography was prepared by mixing 150 gm of silicagel in to a slurry with petroleum ether and pouring the mixture in to the glass column after the adsorbent settled. A cotton plug was kept on the top of the column so that the adsorbent layer is not disturbed during the introduction of the sample and mobile phase. 10 gm of ethanolic extract of *Ipomoea Sepiaria* was charged on the column and eluted successively by gradient elution technique with petroleum ether ,ethylacetate,and methanol. About 20 ml of fractions were collected in a test tube and subjected to TLC in order to identify the presence of compounds. Fractions which gave the same Rf values were pooled together and it was subjected to TLC analysis for the confirmation of the presence of single compound and the results were given in table-XI

TABLE-XI

ISOLATION OF PHYTOCONSTITUENTS FROM ETHANOLIC EXTRACT OF *Ipomoea sepiaria* BY COLUMN CHROMATOGRAPHY

ELUENT	FRACTION NUMBER	NATURE OF RESIDUE	EXAMINATION BY TLC	NUMBER OF COMPOUNDS
PE 100%	1-20	No residue	No spots	No compounds
PE:EA(80:20)	1-12	No residue	No spots	No compounds
	13-19	**Pale green**	**Two spots**	**Two compounds**
	20-35	No residue	No spots	No compounds
PE:EA (60:40)	1-21	No residue	No spots	No compounds
PE:EA(40:60)	1-20	No residue	No spots	No compounds
PE:EA (20:80)	1-22	No residue	No spots	No compounds
EA (100%)	1-14	No residue	No spots	No compounds
	15-21	**Pale green**	**Single compound**	**Single compounds**
	22-35	No residue	No spots	No compounds
EA:MeOH (80:20)	**1-23**	**Pale Yellow**	**Two spots**	**Two compounds**
	24-35	No residue	No spots	No compounds
EA:MeOH (60:40)	1-20	No residue	No spots	No compounds
EA:MeOH (40:60)	1-13	No residue	No spots	No compounds
EA:MeOH (20:80)	1-16	No residue	No spots	No compounds
MeOH(100%)	1-19	No residue	No spots	No compounds

PE-Petether, EA-Ethyl acetate, MeOH-Methanol.

Fraction 13-19 of petroleum ether: ethylacetate (80:20) gave two spots on TLC and these fractions were evaporated to a residue which is called as mixture-I. Further elution with petroleum ether and ethyl acetate with increasing the polarity was not given any compound. Fraction 15-21ofethyl acetate given a single spot on TLC and this fraction was evaporated to a residue which bears pale green colour and its called as compound-III.

Fraction 1-23 of Ethylacetate and methanol (80:20) gave two spots on TLC which was pale yellow in colour and these fractions were evaporated to a residue which was called as mixture-II.

Isolated mixtures such as mixture-I,mixture-II and single compound were given positive reaction for flavonoids by chemical test. Co-TLC examination of mixture II compounds with standard quercetin revealed the presence of quercetin in this mixture.

PURIFICATION AND IDENTIFICATION OF MIXTURE OF COMPOUNDS BY PREPARATIVE THINLAYER CHROMATOGRAPHY:

The mixture of Isolated compounds were isolated by preparative thinlayer chromatography using silica gel G as a stationary phase toluene: ethyl acetate: formic acid,(70:30:0.1) as a mobile phase, UV and ammonia as detecting agent.[74,75,76.] (Fig-13.1)

PREPARATION OF TLC PLATE:

Preaparative TLC plates were prepared for 2mm thickness using silica gel G as stationary phase by using mechanical spreader then the plates were activated at 110ºc for 1 hour in an oven.

SAMPLE APPLICATION:

The isolated compounds were applied on the preparative TLC plate in band form using capillary tube then the plates were placed in TLC chamber.

VISUALISATION OF COMPOUND:

TLC plates were visualized with UV and ammonia in order to identify the spots. Two spots were obtained from each mixture and these spots were scraped carefully to isolate the compounds and the scraped compounds along with silica gel was collected in a 50 ml beaker containing small volume of ethanol. it was filtered to remove the silicagel and the filtrate was evaporated to a residue then it was recrystalized with ethanol.

TABLE-XII

PTLC OF MIXTURE COMPOUNDS

ISOLATED COMPOUNDS	PURIFIED PHYTOCONSTITUENTS	Rf VALUES
MIXTURE-I	Compound I	**0.27**
	Compound II	**0.41**
MIXTURE-II	Compound IV	**0.66**
	Compound V	**0.73**

TABLE-XIII

CO-TLC AND TLC STUDIES OF ISOLATED COMPOUND

Name of the compound	Rf values	Name of the standard	Rf values
Compound IV	**0.66**	Quercetin	**0.66**
Compound III	**0.56**	----	----

FIG-13.1PREPARATIVE TLC OF MIXTURE I AND MIXTURE II

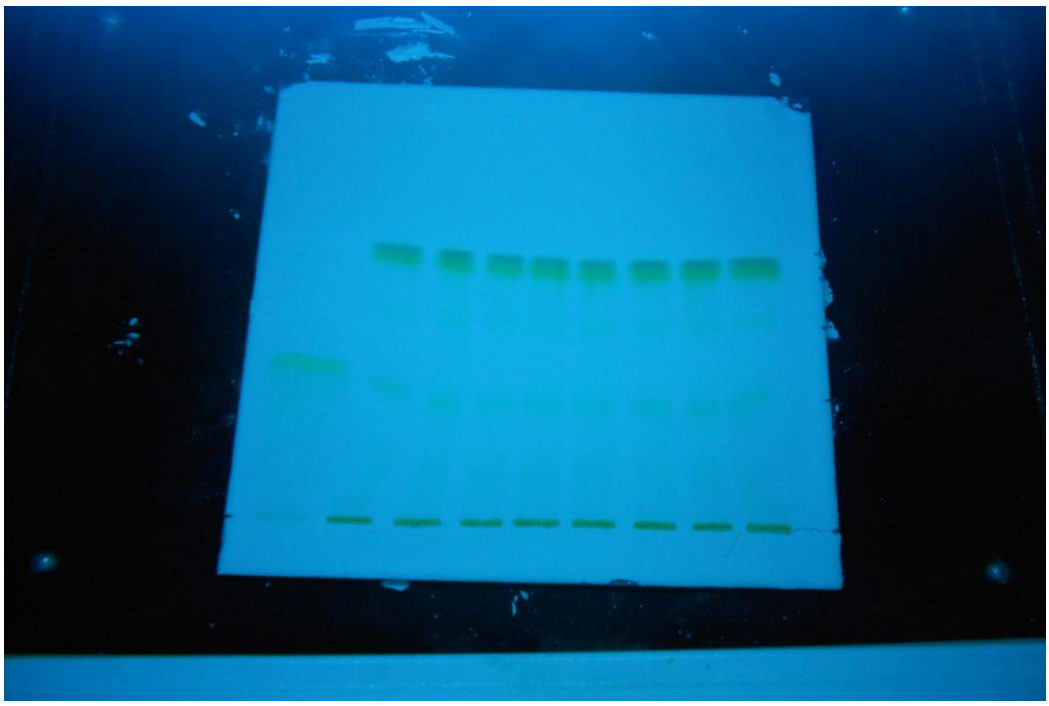


FIG-13.2 CO-TLC WITH QUERCETIN STANDARD

These compounds were further characterized by IR spectrum

TABLE- XIV

COMPOUNDS	FUNCTIONAL GROUP IN cm-1		
	PHENOLIC -OH	C-O Stretching	C === C
COMPOUND-I	3342 cm-1	1392 cm-1	1598 cm-1
COMPOUND-II	3601 cm-1	1445 cm-1	1592 cm-1
COMPOUND-III	3601 cm-1	2927 cm-1	1594 cm-1
COMPOUND-IV	3371 cm-1	1408 cm-1	1590 cm-1
COMPOUND-V	3296 cm-1	2926 cm-1	1584 cm-1

Compound-I ,compound-II, compound-IV and V were isolated by preparative TLC . A single compound has already been identified as compound III, These isolated compounds were given positive reaction for flavanoids.CO-TLC examination of compound IV with standard quercetin revealed that the compound IV may be quercetin. (Fig-13.2)

CHAPTER-VI

PHARMACOLOGICAL SCREENING

SECTION-A

IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF

Ipomoea sepiaria

. Majority of the present day, diseases are due to the shift in the balance of pro-oxidant and antioxidant. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of the current life (or) If not effectively scanned by cellular constituents and depletion of the dietary antioxidant may leads to various disease conditions, such as rheumatoid arthritis, haemorrhagic shock, CVS disorders and neurodegenerative disease.

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Oxidative stress causes various disease conditions such as ageing, anaemia, arthritis, asthma, inflammation, ischemia, neurodegeneration, Parkinson's disease, and perhaps dementia. Phenolic hydoxyl group compounds may exert powerful antioxidant activity *invitro* by lipid peroxidase inhibitors or scavenging the free radicals. The antioxidant activity can be performed by various methods.

a. DETERMINATION OF RADICAL SCAVENGING ACTIVITY AGAINST HYDROGEN PEROXIDE:

PRINCIPLE:

The radical scavenging activity against hydrogen peroxide of plant extract was determined by using the method of Ruch *et al.* The principle based o the capacity of the extracts to decompose the hydrogen peroxide to water.[83]

INSTRUMENT:

Shimadzu UV Visible spectrophotometer, Model 1800.

REAGENTS:

6% hydrogen peroxide diluted with water in the ratio of 1:10.

0.1M,P^H 7.4 phosphate buffer.

PROCEDURE:

The ethanolic extract of *Ipomoea sepiaria* was dissolved in ethanol to get a stock of 1mg/ml. Varying quantities of the stock solution was added to 3.8ml of 0.1M phosphate buffer solution (P^H 7.4) and then mixed with 0.2ml of hydrogen peroxide solution. The absorbance of the reaction mixture was measured at 230nm after 10min.The mixture without sample was used as blank. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula %inhibition = (A control-A sample)/Acontrolx100.The results are present in Table. XV

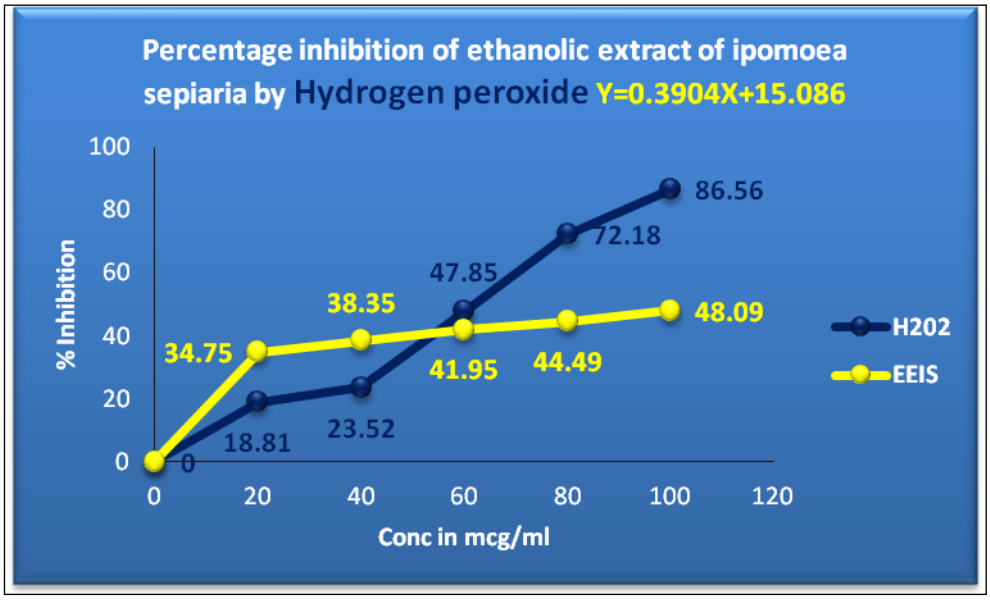
TABLE-XV

HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *Ipomoea Sepiaria*

S. No.	**Conc. in µg/mL**	**Percentage inhibition by standard Hydrogen peroxide**	**Percentage inhibitionby EEIS**
1	20	18.81	34.75
2	40	23.52	38.35
3	60	47.85	41.95
4	80	72.18	44.49
5	100	86.56	48.09
	IC_{50}	47.04 **µg/ml**	89.43

Fig:14 Hydrogen peroxide scavenging activity of Ethanolic extract of

Ipomoea sepiaria



b. DPPH SCAVENGING (2, 2-diphenyl-1-picrylhydrazyl) ASSAY

Principle

The free radical scavenging activity is measured by using DPPH assay. DPPH molecules are stable free radicals that contain unpaired electron. The quantitative estimation of free radical scavenging activity is determined according to Blois *et al* method. When the solution of DPPH is mixed with substance that can donate a hydrogen atom, it is converted to its reduced form which is indicated by the colour changes from deep violet to pale yellow colour. The result of the antioxidant activity is expressed as EC_{50} or IC_{50}.

Materials required

1. Ethanolic leaf & Stem extract of Ipomoea sepiaria
2. DPPH
3. 95% ethanol
4. Ascorbic acid

Procedure

The DPPH solution (0.1mM) in ethanol was prepared and 1.0ml of this solution was added to 3.0 ml of extract solution (or standard) in water of different concentrations (10-50 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Scavenging of DPPH radical was calculated using the following equation.

$$\% \text{ inhibition} = A_0\text{-}A_1/A_0 \times 100$$

Where A_0-The absorbance of the control reaction

A_1- The absorbance of the test sample

IC_{50} (concentration providing 50% inhibition) was calculated graphically using a calibration curve by plotting the extract concentration Vs % inhibition. The mean values were obtained from triplicate experiments.

TABLE-XVI

DPPH RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *Ipomoea Sepiaria*

Conc.µg/ml	**STANDARD %inhibition**	**EEIS %inhibition**
10	39.94	30.69
20	47.05	36.76
30	70.94	39.12
40	88.98	41.82
50	96.94	43.50
IC_{50} values	17.9625 µg/ml	99.80 µg/ml

Values are expresses mean ± SEM (n=3)

FIG:15 Hydrogen peroxide scavenging activity of Ethanolic extract of

Ipomoea sepiaria

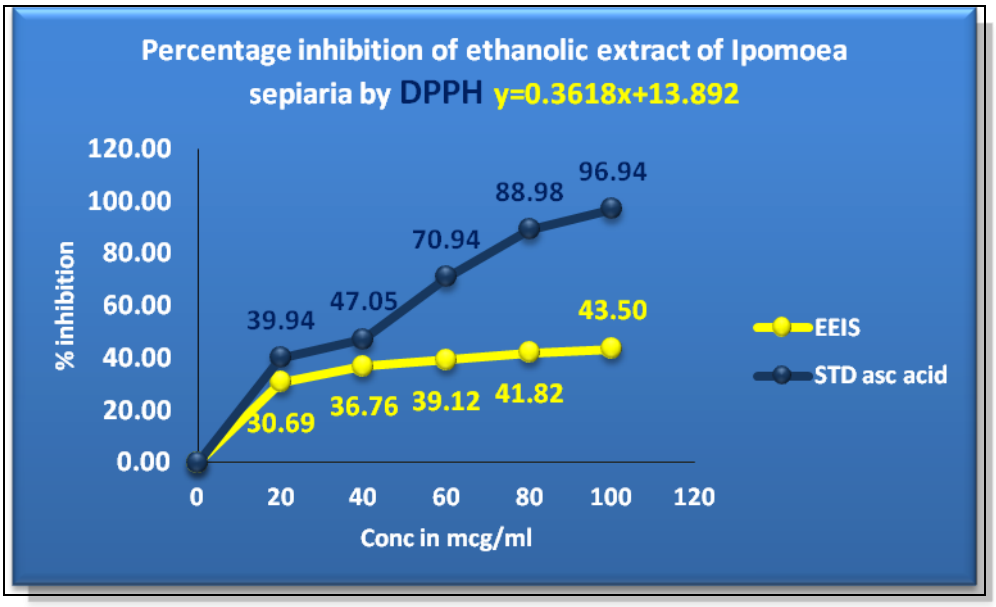


TABLE-XVII

DPPH ANTIOXIDANT ACTIVITY OF ISOLATED COMPOUNDS

Conc.µg/ml	STANDARD %inhibition	MIXTURE-I COMPOUND	MIXTURE-II COMPOUND	SINGLE COMPOUND
10	39.94	12.32	48.77	22.65
20	47.05	25.13	53.14	31.34
30	70.94	37.55	62.55	45.44
40	88.98	50.33	69.94	56.11
50	96.94	64.55	81.53	68.43
IC_{50} values	17.9625 µg/ml	77.23 µg/ml	46.13 µg/ml	69.43 µg/ml

Fig:16 DPPH scavenging activity of Isolated mixture-I

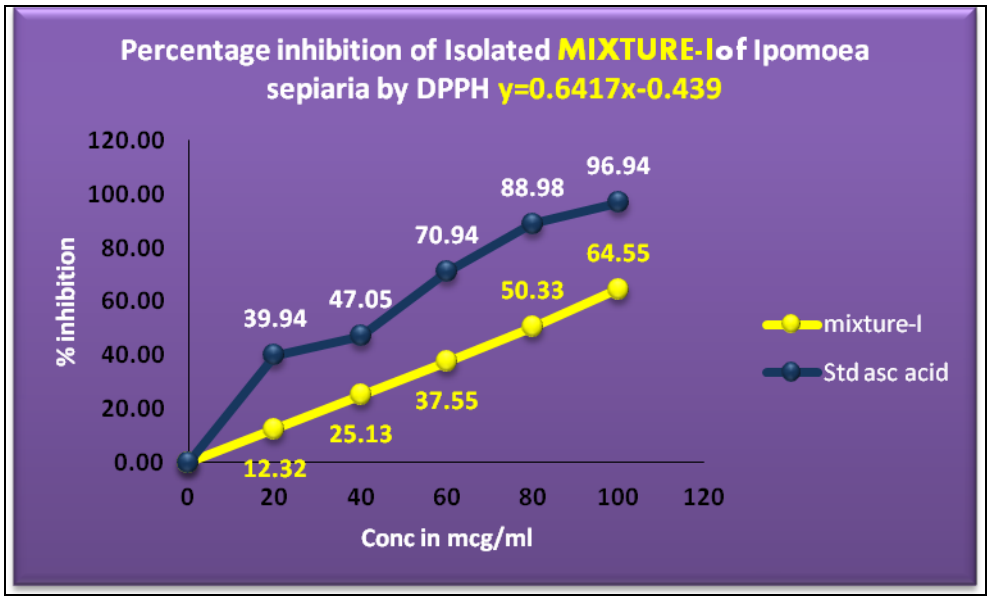


Fig:17 DPPH scavenging activity of Isolated mixture-II

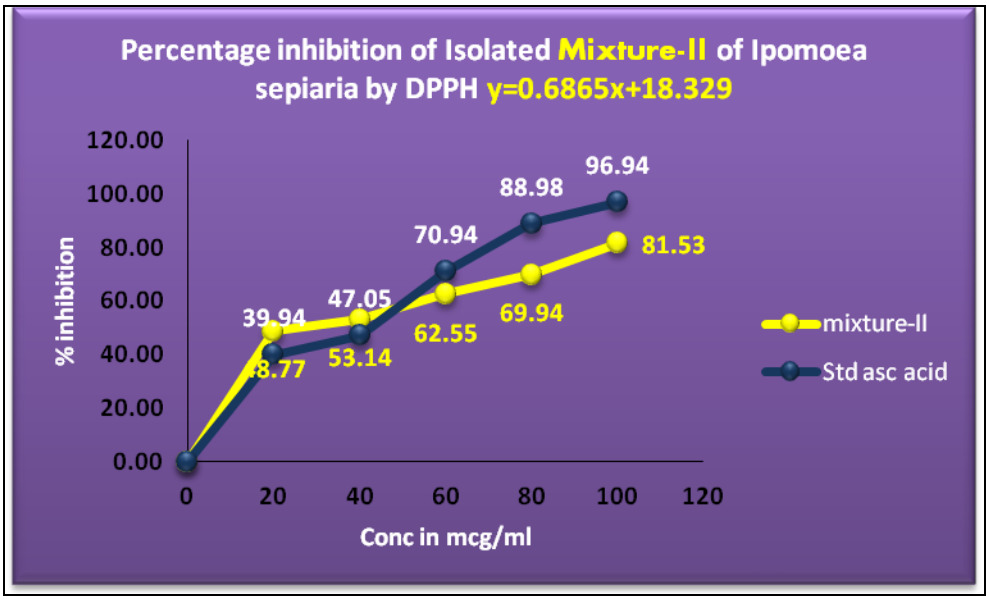
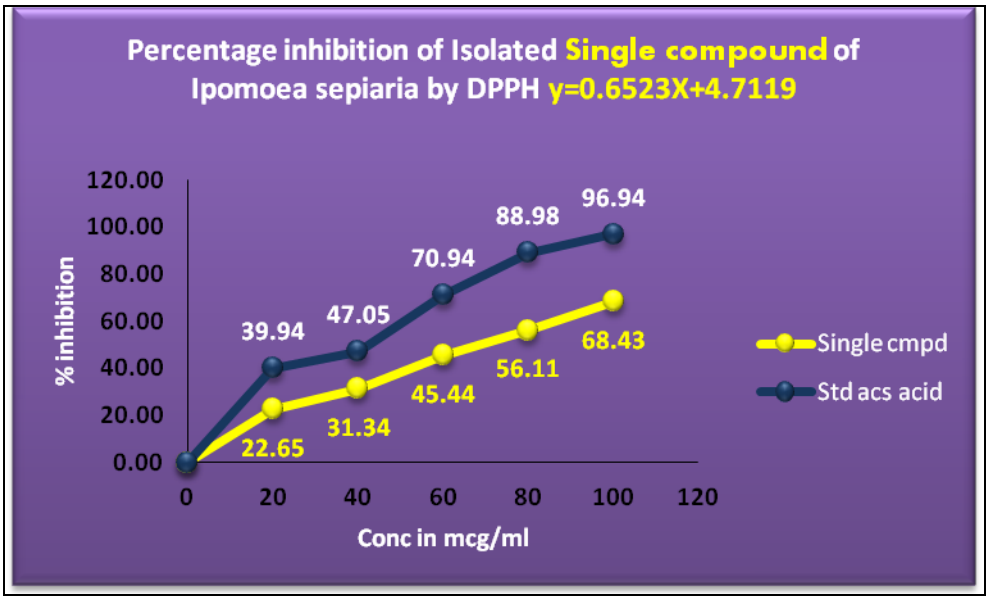


Fig:18 DPPH scavenging activity of isolated Single compound



Mixture II exhibited significant antioxidant activity than mixture I and single compound, mixture two contains flavonoids evaluated against acrylamide induced neuropathic pain in rats.

SECTION-B

PHARMACOLOGICAL EVALUATION OF *Ipomoea Sepiaria* ON ACRYLAMIDE INDUCED NEUROPATHIC PAIN IN RATS [78,80,81,82,94,95]

Neuropathic pain associated with peripheral nerve injury which is characterized by the sensory abnormalities such as unpleasant and abnormal sensation (dysesthesia), an inceased response to painful stimuli (hyperalgesia), and pain in response to a stimulus that does not normally provoke pain (allodynia) According to ethanomedical claim leaf decoction of Ipomoea sepiaria is used for nervous disorders. Hence the Ethanolic extract of Ipomoea sepiaria has been evaluated for acrylamide induced painful neuropathy in rats.

Materials and Methods

Plant Material

Ethanolic leaf extract of ***Ipomoea sepiaria,*** **[EEIS]**

Animals

Wistar rats (either sex, 150-200gm) were employed in the present study. Animals were maintained at laboratory diet and allow to free access to water ad libitum. They were housed in the animal house and exposed to normal cycle of light and dark. The experimental protocol was duly approved by the institutional animal ethical committee and animals were maintained as per CPCSEA guideline. (Committee for the purpose of control and supervision of experiments on Animals Ref no:14024/E1/4/2011) of the Dean, Madurai medical college, Madurai.

Chemicals

Acrylamide was purchased from CDH, chemicals New Delhi. All other chemicals used for this study were of analytical grade.

Induction of Neuropathic pain

The Acrylamide 30mg/kg was administered by intraperitonially once in three days, for 24 consecutive days to induce painful neuropathy. All the groups of animals were subjected to sensory behavioral tests such as paw cold allodynia, hot plate test, and tail immersion test in order to assess the degree of nociceptive threshold on certain day intravels, ie.0,3, 6,9, 12,15, 19,21 & 24^{th}day.

Experimental Design

Six groups, each comprising six Wistar rats, were employed in the present study.

Group I (Normal control group)

Rats were not subjected to administration of vehicle and acrylamide and were kept for 24 days. Behavioural tests were employed to assess nociceptive threshold on different day's i.e. 0,3, 6,9, 12.15,19,21 & 24^{th}. All the animals were sacrificed according to CPCSEA guidelines at the end of the 24^{th} day. Sciatic nerve of the animals were isolated and homogenated to estimate biochemical markers such as TBARS, reduced glutathione, total protein and total calcium levels. Histopathological studies also carried out with the distal portion of the sciatic nerve.

Group II (Acrylamide 30mg/kg, i.p)

Acrylamide (30mg/kg, i.p) was administered to normal rats once in three days for 24 consecutive days. Behavioural tests and Biochemical parameters were assessed as described in group I.

Group III (Ethanolic extract of EEIS 100mg/kg treated group)

Ethanolic leaf extract of *Ipomoea sepiaria* 100mg/kg, p.o was administered two hours before each acrylamide injection (acrylmide was administered once in three days) for

24 consecutive days. Behavioural tests and Biochemical parameters were assessed as mentioned in group I.

Group I V (Ethanolic extract of EEIS 200mg/kg treated group)

Ethanolic leaf extract of *Ipomoea sepiaria* 200mg/kg,p.o, was administered two hours before each acrylamide injection (Acrylmide was administered once in three days) for 24 consecutive days. Behavioral tests and Biochemical parameters were assessed as mentioned in group I.

Group V (Isolated compound (mixture-II) of EEIS 5mg/kg treated group)

Ethanolic leaf extract of *Ipomoea sepiaria* 5 mg/kg,p.o was administered two hours before each acrylamide injection (Acrylmide was administered once in three days) for 24 consecutive days. Behavioural tests and Biochemical parameters were assessed as mentioned in group I.

Group VI (Pregabaline 10mg/kg, treated group)

Pregabaline (10mg/kg, p.o) was administered two hours before each acrylamide injection (acrylmide was administered once in three days) for 24 consecutive days. Behavioral tests and Biochemical parameters were assessed as mentioned in group I.

SENSORY BEHAVIOURAL ASSESMENT

Paw cold allodynia

Cold allodynia of the hind paw was assessed using acetone drop method as described by Choi et al (1994) with slight modification, Assessing the reactivity to non-noxious cold chemical stimuli.the rats were placed on the top of a wire mesh grid allowing access to the hind paw.Acetone(0.1ml) was sprayed on the plantar surface of hind paw of

rat and time taken for the withdrawl of the hind paw from the mesh surface was noted ,with cut-off time of 60 seconds. [85]

Hot plate test

Heat thermal sensitivity of the hind paw was assessed by using Eddy's hot plate as described method of Eddy et at, with slight modification for assessing the degree of noxious thermal sensation. The rats were placed on the top of a preheated (52 ± 0.5ºc) hot plate surface, allowing access to the hind paw withdrawal response to degree of the nociceptive threshold. The cut- off time of 20 seconds was maintained.[84]

Tail immersion test

Tail immersion test was carried out to assess the spinal heat thermal sensitivity. Tip of the rat's tail was immersed in heat noxious temperature (52 ± 0.05ºc) till the tail was withdrawn. Thermal heat hyperalgesia was assessed by duration of the tail withdrawal reflex. The cut off time of 10 s was maintained.[86]

Biochemical estimation of markers of oxidative stress

All the groups of animals were sacrificed after 24th days by cervical dislocation and the sciatic nerve was isolated immediately and used for the biochemical estimation. The distal most part of the nerve, which was used for histopathological study. Freshly excised sciatic nerve homogenate (10%) was prepared with 0.1M Tris Hcl buffer(PH-7.4) and the homogenate was kept in ice water for 30 min and centrifuged at 4ºc (2000g, 10 min). The supernatant of homogenate was separated and which was used to estimate following biochemical markers.[93]

Estimation of total protein content

The protein concentration was estimated according to the method of Lowry *et al* [s]using bovine serum albumin as a standard. The absorbance was determined spectrophotometrically at 750nm.[92]

Estimation of total calcium

Total calcium level was estimated in sciatic nerve as described method of Severtnghaus and Ferrebee and Muthuraman.Total calcium level was estimated in sciatic nerve. The sciatic nerve homogenate was mixed with 1ml of trichloroacetic acid (4%) as in ice cold condition and centrifuged at 1500g for 10 mins. The clear supernatant was used for the estimation by atomic emission spectroscopy at 556 nm.[89,90]

Estimation of reduced glutathione

Equal quantity of sciatic nerve homogenate was mixed with 10% trichloroacetic acid and the mixture was centrifuged to separate proteins. To 0.01ml of this supernatant, 2ml of phosphate buffer (PH-8.4), 0.5ml of 5, 5' dithiobis (2-nitrobenzoic acid) and 0.4ml of distilled water were added. Mixture was vortexed and the absorbance was taken at 415nm within 15mins. The concentration of reduced glutathione was expressed as µg/mg of protein.[88]

Estimation of TBARS

The thiobarbituric acid reactive substances (TBARS) level was estimated as per the spectrophotometric method described by Ohkawa *et al* method, To each test tube, 0.5 ml of supernatant,0.5 ml normal saline,1 ml of 20% trichloroacetic acid (TCA) and 0.25 ml of TBA reagent (200 mg of thiobarbituric acid in 30 ml distilled water and 30 ml of acetic acid) were added. The test tubes were kept for boiling at 95º C for one hour. To each test tube, 3 ml of n-butanol was added and mixed well. These test tubes were centrifuged at 3000rpm for 10 minutes. The separated butanol layer was collected and read in a

spectrophotometer against blank at 535 nm. Concentration of thiobarbituric reactive substances was expressed in terms of nmol of malondialdehyde per mg of protein.[87]

`

TABLE-XVIII

Effects of Ethanolic extract of *Ipomoea sepiaria* on tissue biochemical changes

Groups	TBARS (nmol/mg of protein)	GSH (µg/mg of protein)	Total calcium (ppm/mg of protein)
Normal	13.20 ± 0.23	69.37 ± 2.53	1.93 ± 0.79
Acrylamide	56.41 ± 0.70 [a]	12.64 ± 3.15 [a]	16.87 ± 1.31 [a]
EEIS (100 mg/kg)	29.2 ± 0.82 [b]	31.78 ± 2.96 [b]	7.16 ± 1.04 [b]
EEIS (200 mg/kg)	21.2 ± 0.85 [b]	54.63 ± 3.94 [b]	5.48 ± 1.17 [b]
EEIS-C (5 mg/kg)	20.55 ± 0.33 [b]	59.51 ± 2.76 [b]	3.64 ± 0.95 [b]
Pregabalin	15.13 ± 0.51 [b]	61.64 ± 3.36 [b]	2.72 ± 0.86 [b]

Data were expressed as mean ± SD (n=6).

EEIS,(Ethanolic extract of ipomoea sepiaria); EEIS-C,(Ethanolic extract of ipomoea sepiaria isolated compound);

[a]*P < 0.05* Vs. normal group.

[b]*P < 0.05* Vs. acrylamide control group.

Digits in parentheses indicate dose in mg/kg.

TBARS-Thiobarbituric acid reactive substance; GSH-Reduced glutathione; Ipomoea sepiaria,

Histopathological examination

Samples of sciatic nerve were fixed with 10% formalin and cut in to sections with 4µm thickness, staining was done by using haematoxylin and eosin. Nerve sections were analysed qualitatively under light microscope (45x) for axonal degeneration and fibres dearrangements.Effect of *Ipomoea sepiaria* on acrylamide induced histopathological changes were shown in Fig-19 to 23. [91]

Fig-19 NORMAL CONTROL GROUP-I

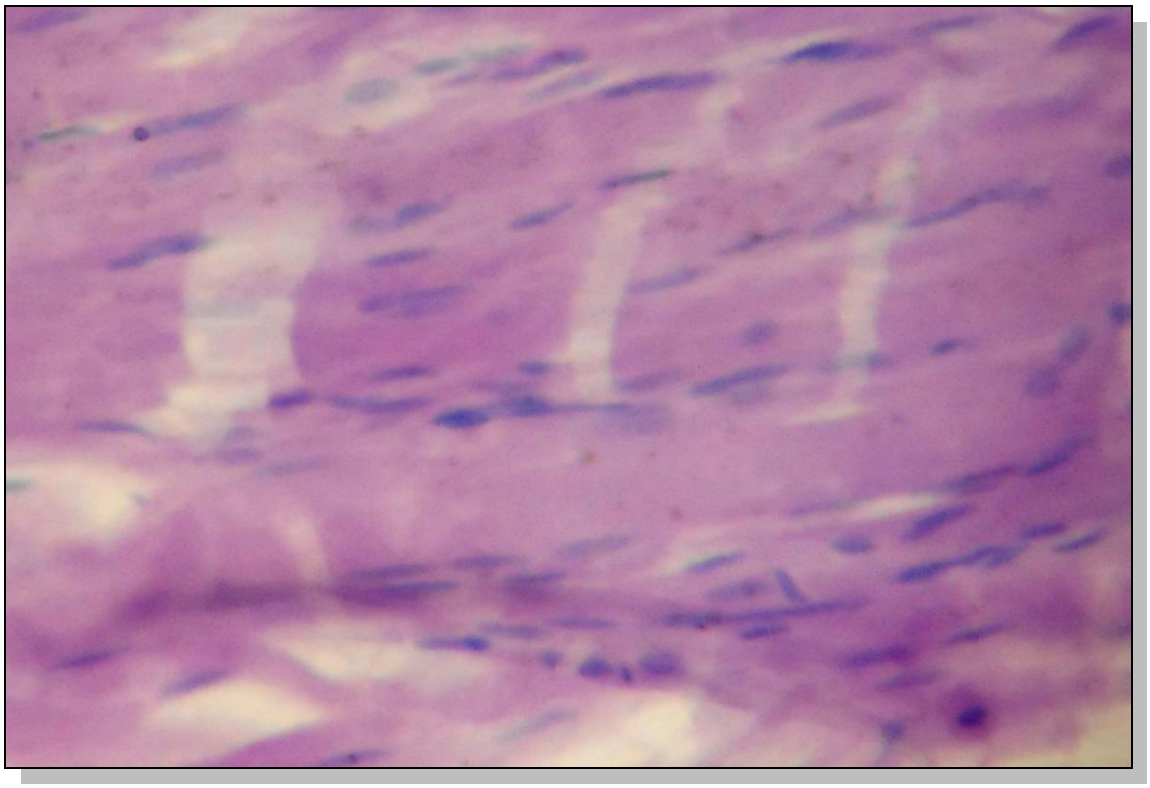


Fig-20 ACRYLAMIDETREATEDGROUP-II

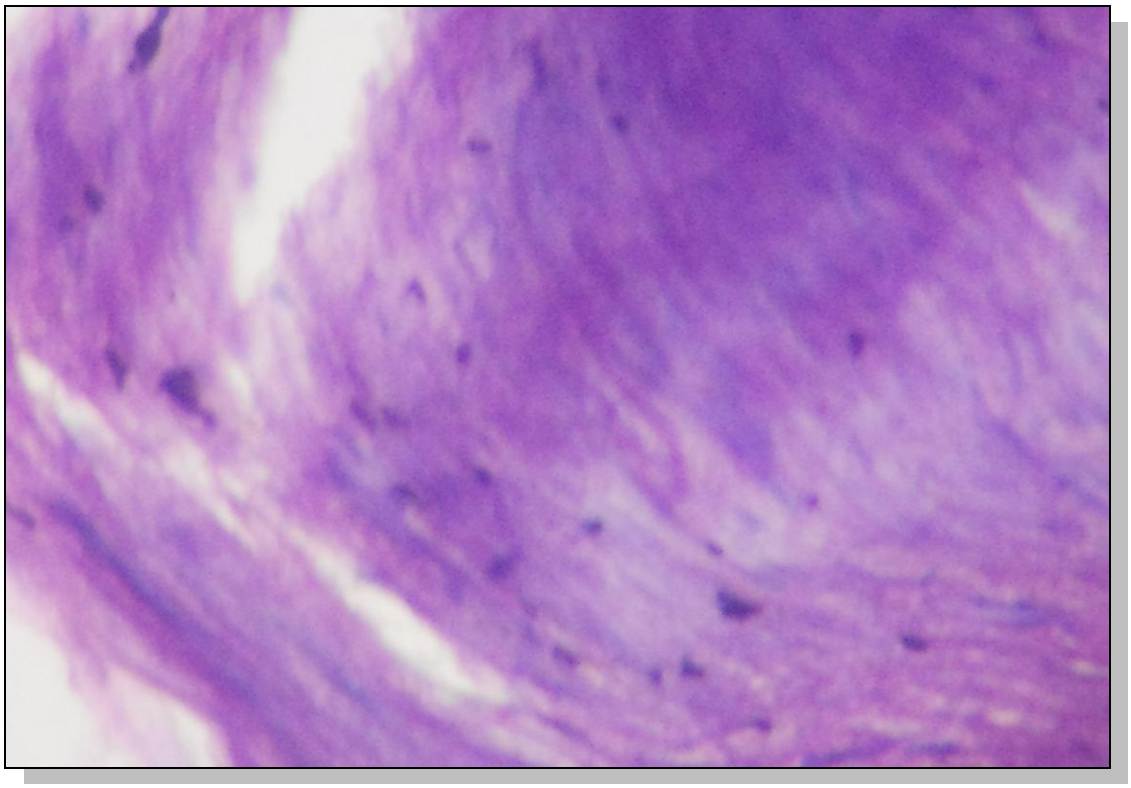


Fig-21 EEIS TREATMENT 100 mg/kg GROUP-III

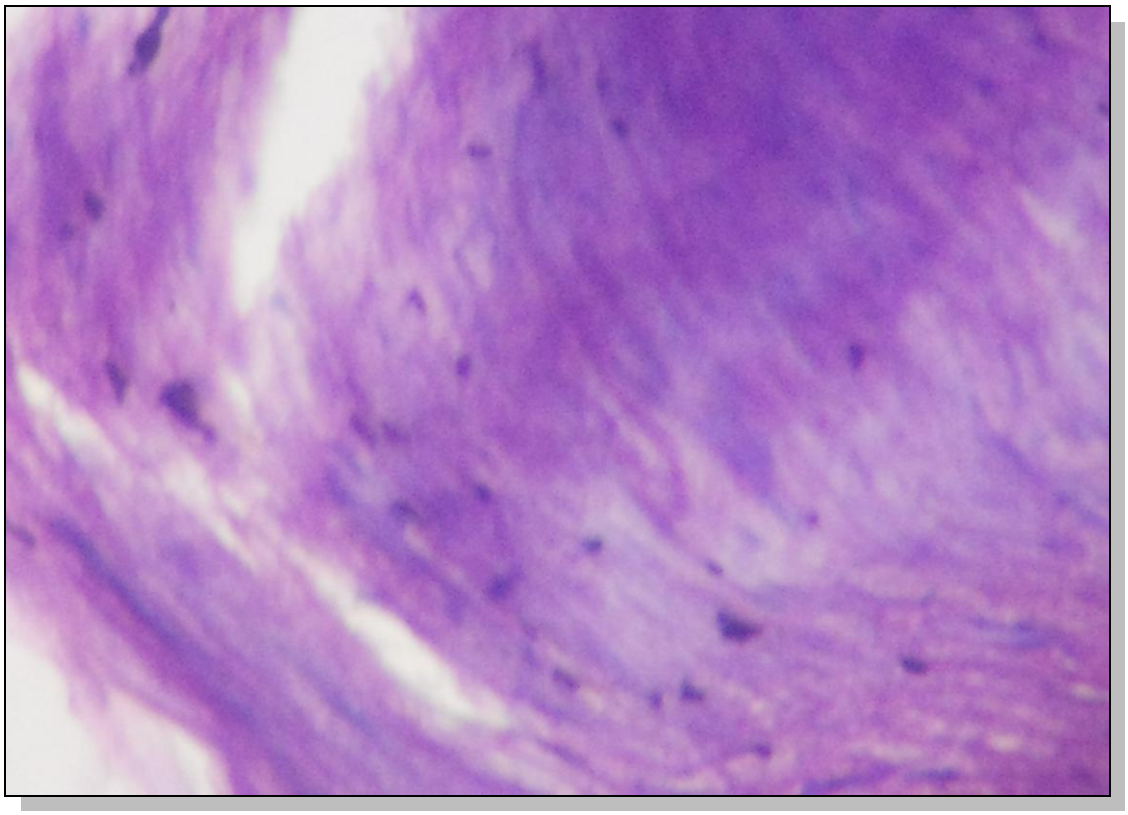


Fig-22 EEIS TREATMENT 200 mg/kg GROUP-IV

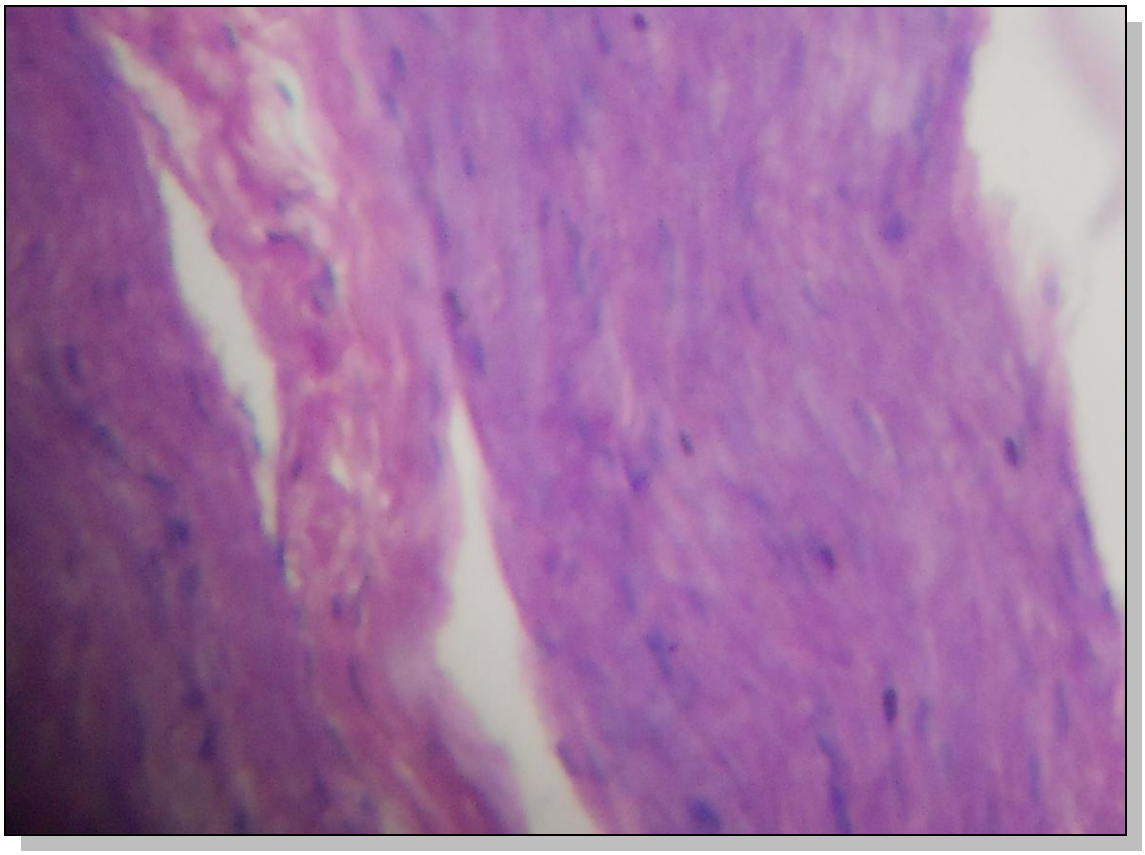


Fig-23 EEIS ISOLATED COMPOUND TREATMENT 5 mg/kg GROUP-V

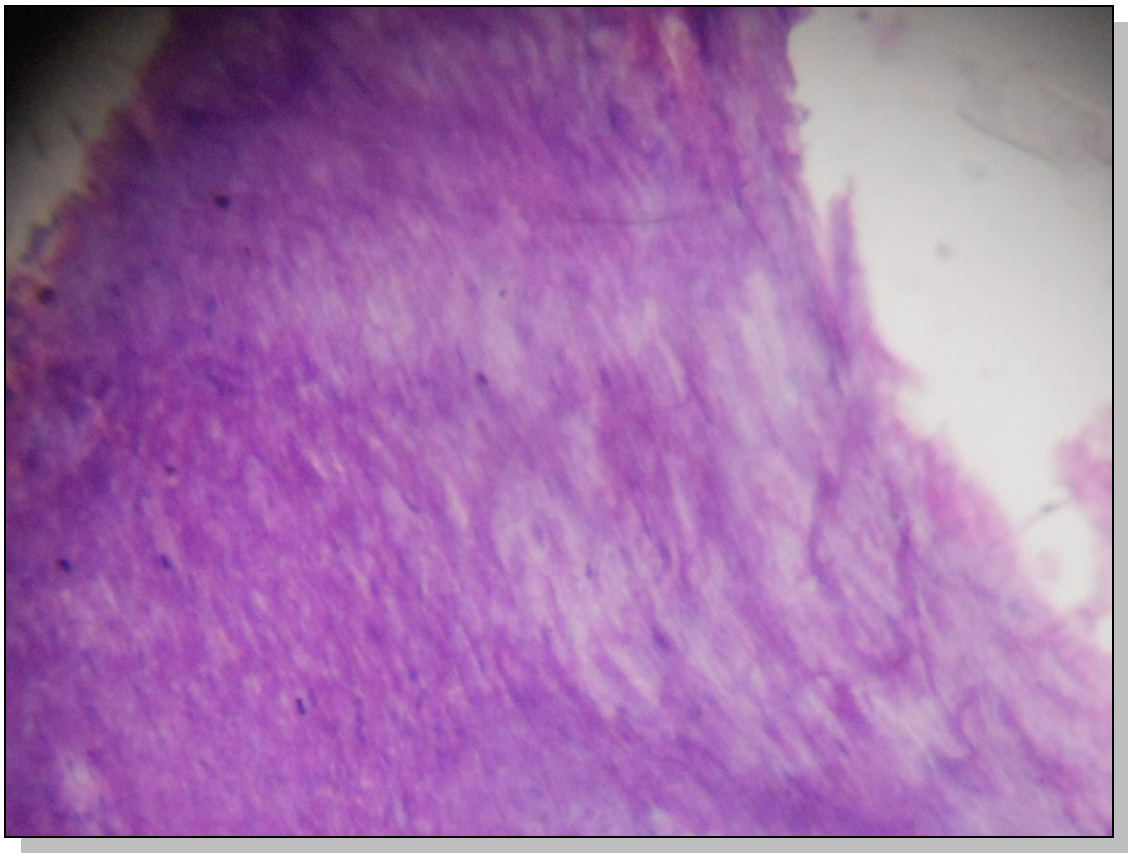


Fig 19-23 shows cross section of sciatic nerve of normal acrylamide, EEIS 100 mg, 200 mg and 5 mg/kg.p.o, Treated groups respectively. Fig-19 showed normal histopathology of sciatic nerve, In fig-20 shows acrylamide induced axonal degeneration and dearrangement of nerve fibres. Fig-21, 22 and 23 pretreatment of *Ipomoea sepiaria* showed decrease in the acrylamide induced histopathological changes of sciatic nerve.

Statiscal analysis

All the results were expressed as standard error of mean (SEM). Data obtained from behavioural tests were statistically analysed by using two-way repeated ANOVA, while data of biochemical parameters were analysed using one way ANOVA. In both cases, Tukey's multiple range tests were applied for post-hoc analysis. A value of $p<0.05$ was considered to be statistically significant.

SECTION-C

IN VITRO ANTI CANCER ACTIVITY

The human liver cancer cell line (HepG2) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week,Ethanolic extract of ipomoea sepiaria was used in five different concentrations such as 31.25µg,62.5 µg,125 µg,250 µg, and 500 µg.[96,97]

CELL TREATMENT PROCEDURE

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 31.25µg,62.5 µg,125 µg,250 µg, and 500 µg, The final volume in each well was 200 µl and the plates were incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT ASSAY

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using GraphPad Prism software.

Fig- 24: CONTROL

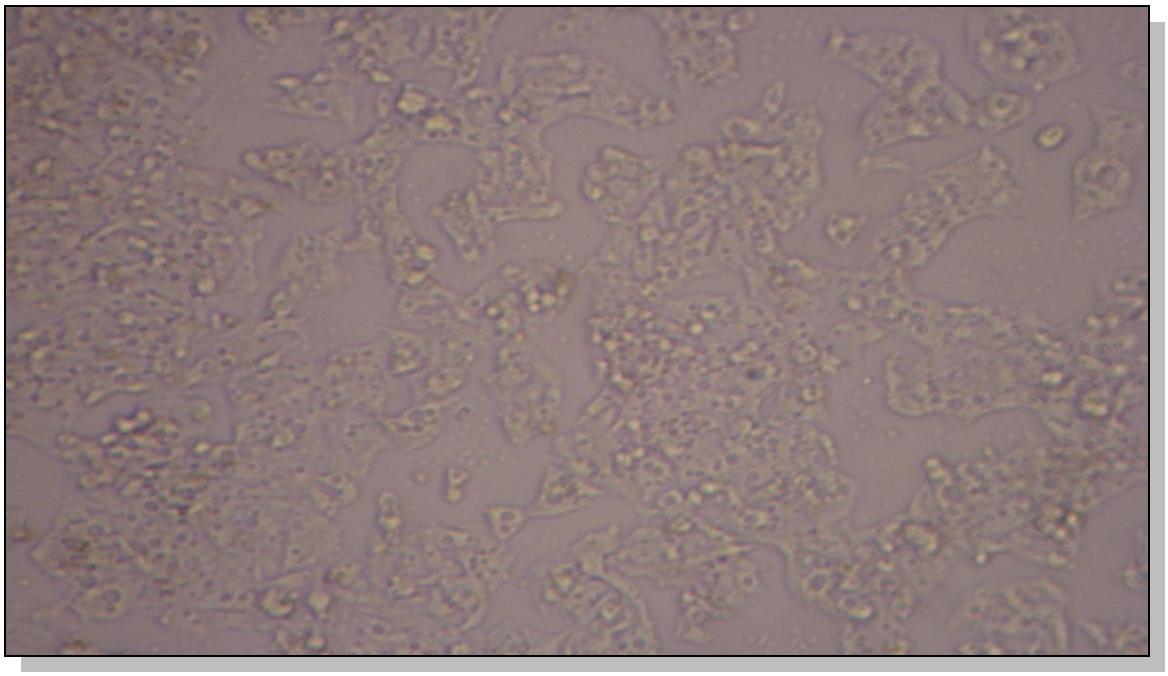


Fig 25: 31.25 µg OF ETHANOLIC EXTRACT OF *Ipomoea sepiaria* ON LIVER CANCER CELLS

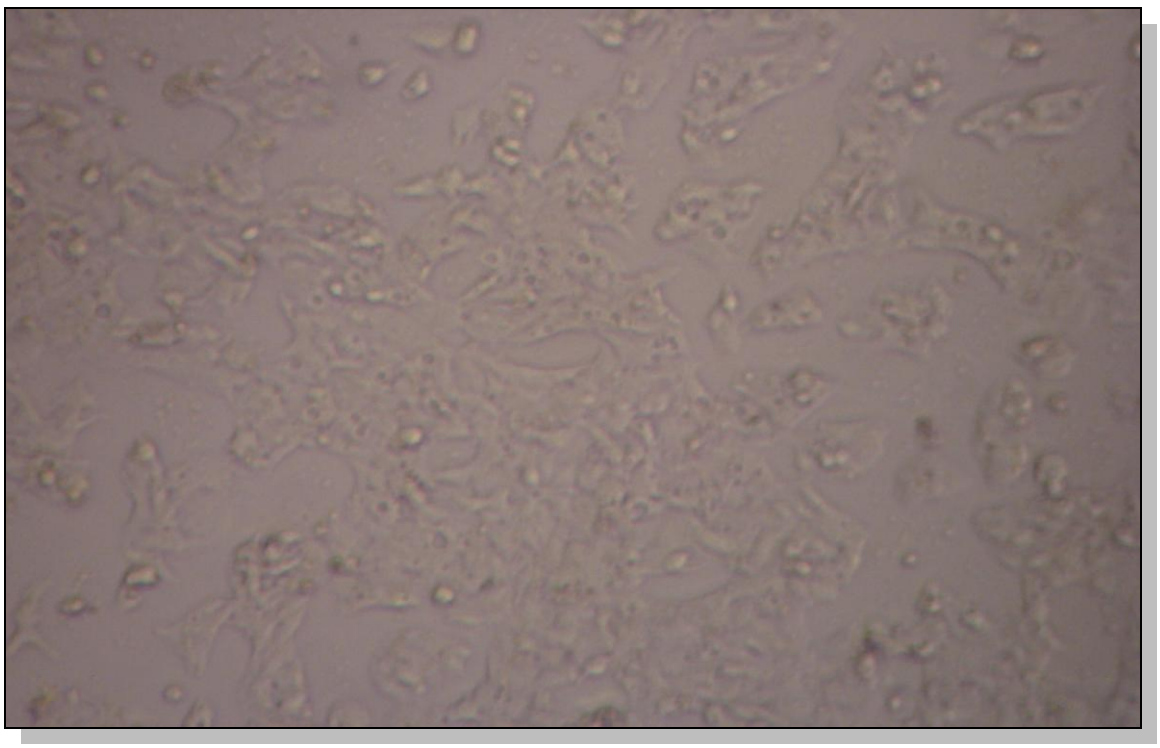


Fig-26: 62.5 µg OF ETHANOLIC EXTRACT OF *Ipomoea sepiaria* ON LIVER CANCER CELLS

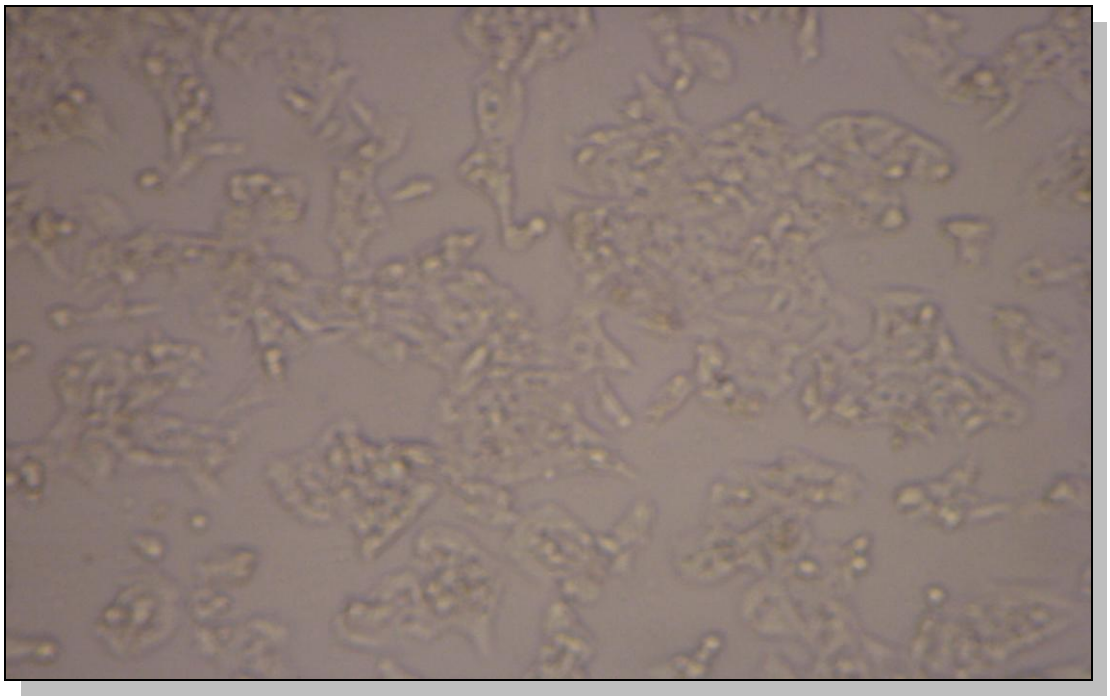


Fig-27: 125 µg OF ETHANOLIC EXTRACT OF *Ipomoea sepiaria* ON LIVER CANCER CELLS

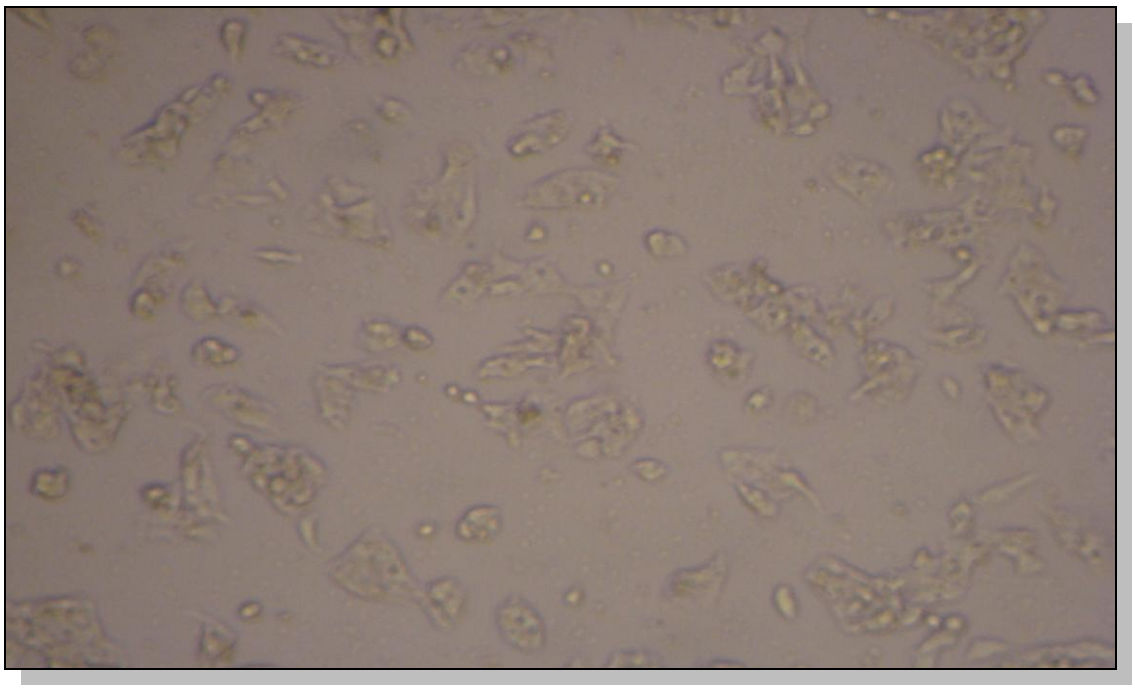


Fig 28: 250 µg OF ETHANOLIC EXTRACT OF *Ipomoea sepiaria* ON LIVER CANCER CELLS

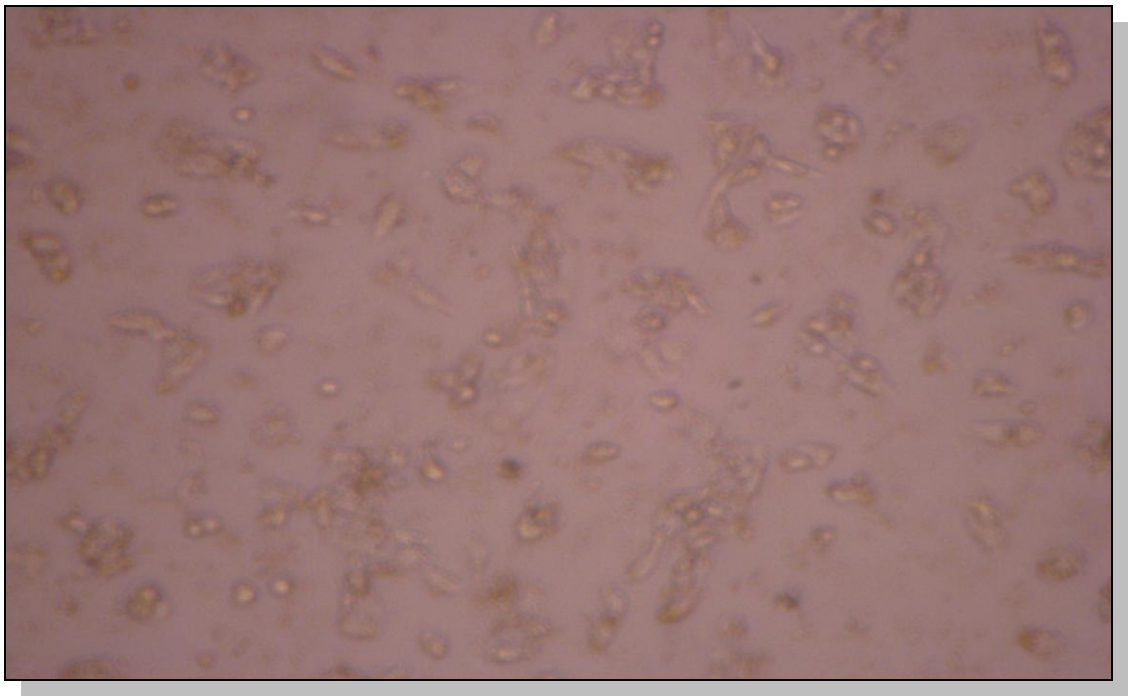


Fig-29: 500 µg OF ETHANOLIC EXTRACT OF *Ipomoea sepiaria* ON LIVER CANCER CELLS

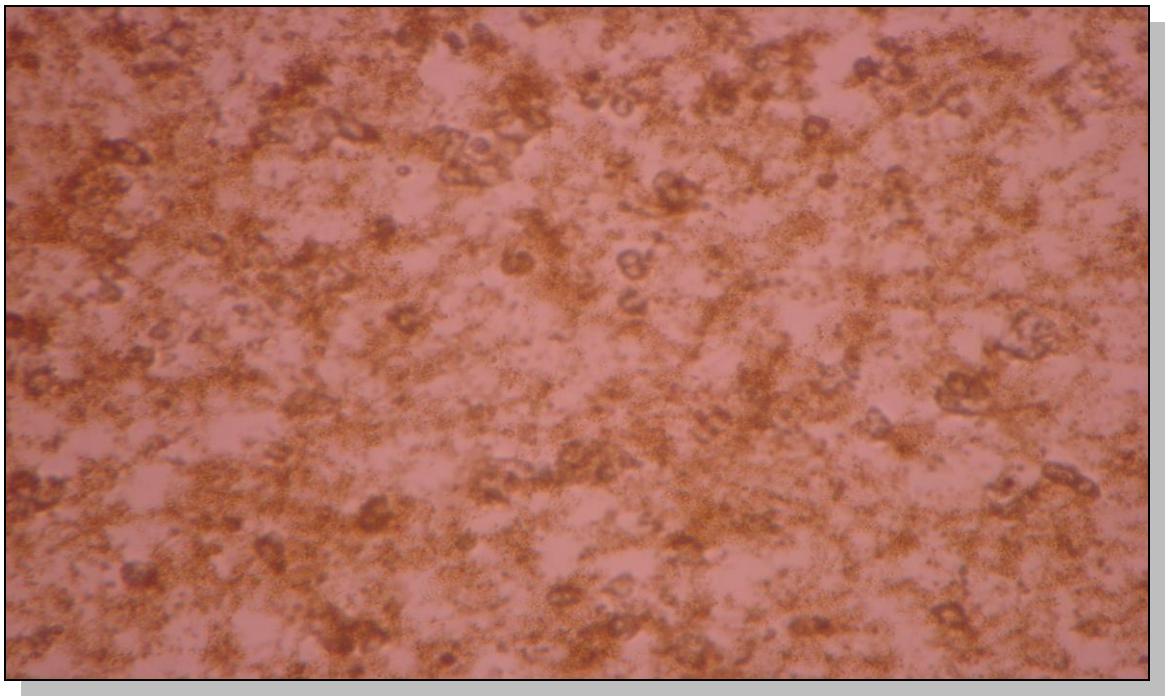


TABLE-XIX

ETHANOLIC EXTRACT OF *Ipomoea Sepiaria* on HepG2 (LIVER CANCER)

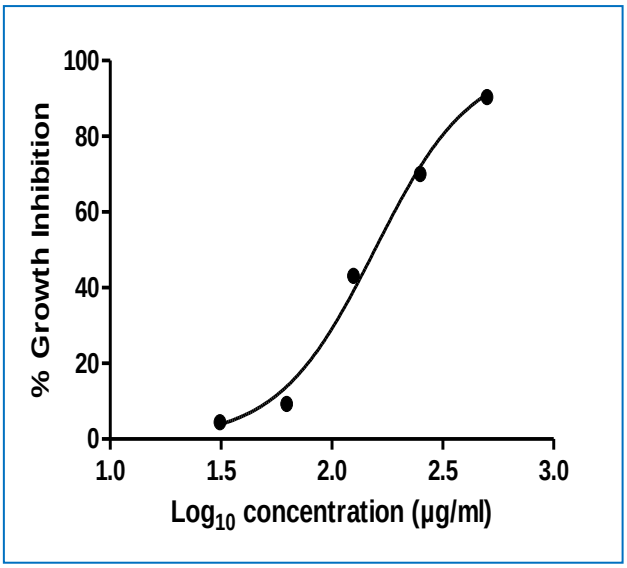
CELL LINES

HepG2							
	Conc	31.25 µg	62.5 µg	125 µg	250 µg	500 µg	Cont
	ABS	0.428	0.408	0.255	0.144	0.052	0.455
		0.419	0.411	0.242	0.148	0.037	0.432
		0.433	0.396	0.265	0.11	0.04	0.453
S	Avg	0.426	0.405	0.254	0.134	0.043	0.446

IC 50 **156.1 ug/ml**

R^2 **0.9926**

Conc of EEIS (µg)	% Cell Inhibition
31.25	4.47
62.5	9.32
125	43.13
250	70.01
500	90.37



CHAPTER-VII

RESULTS AND DISCUSSION

This dissertation covers the works on pharmacognostic, phytochemical and pharmacological studies on the leaves of *Ipomoea sepiaria* belongs to the family convulvulaceae.

CHAPTER-I

Introduction of this dissertation gives the information about the antioxidant approach of herbal medicine, neuropathy and its etiology,causes of neuropathy pathophysiology, and hepatocellular carcinoma including herbal medicine cures for both neuropathy and hepatocellular carcinoma . Acrylamide as a neurotoxin to induce neuropathic pain and animal models of neuropathic pain were also discussed in this chapter.

CHAPTER-II

Literature survey pertaining to the pharmacognostic, phytochemical and pharmacological studies and ethanomedical information of *Ipomoea sepiaria* were given in this chapter.

CHAPTER-III

Aim and scope of the dessertation have been discussed in this part of the study.

CHAPTER-IV

Pharmacognostic studies

Pharmacognostic standards for the leaves of *Ipomoea sepiaria* which includes the macroscopical, microscopical, quantitative microscopy and physical standards were discussed in this chapter.

Section-A

Scientific classification, synonym, vernacular names,geographical distribution, Habit and Habitat of the plant, and morphological characters of leaves, Flowers, fruits and seeds of this plant were dealt in this part of the study,

Salient features of the macroscopy of leaves were observed, such as simple, alternate, margin is entire and the shape is ovate. Leaflet venations are reticulate and the leaves are dark green in colour having 1.5 inch long.

Section-B

It deals with the microscopical studies of the leaves to find out the arrangement of tissues,

The important anatomical features observed in the leaves of *Ipomoea sepiaria* are as follows.

- The stomata are exclusively paracytic type.Each stoma has two subsidiary cells lying parallel to the guard cells. The stomata are elliptical in shape measuring 20x30µm in size.
- The venation pattern is less densely reticulate and the veins are thin and undulate.
- The vein terminations are long,slender,curved and simple. Most of the terminations bud in a cluster of terminal-tracheids (fig.7.2). The tracheids are rectangular and square shaped with spiral thickenings.

The following anatomical characters were observed with powder microscopy of leaves reveal the presence of,

- Glandular trichomes are present along with epidermal cells which consist of a circular plate of glands comprising about eight triangular cells.
- The stomata are paracytic type and the subsidiary cells are mostly unequal in size (fig.12.2). The surface of the cells appear finely dotted cuticular markings. **(fig.10.1,2.11.1,2)**

Section-D

This part of study deals with the determination of leaf constants, such as vein-islet number, vein termination number, stomatal number and stomatal index, The results were presented in table.

LEAF CONSTANTS

Content	Leaves		
	Min	Ave	Max
Vein islet No	7	8	10
Vein termination No	6	8	9
Stomatal number	23	29	34
Stomatal index	5.88	11.46	17.95

Section-E

Physical parameters like profile of ash values, loss on drying and various extractive values have been determined for this plant which are given below.

PHYSICAL PARAMETERS

Content	Leaves and stem % w/w		
	Min	Ave	Max
Total ash	14.92	15.69	16.88
Acid insoluble ash	5.65	6.55	6.82
Water soluble ash	9.83	6.82	10.14
Loss on drying	5.92	6.26	6.45

EXTRACTIVE VALUES

S.NO	Solvents used	Extractive values %
1	Petroleum ether	4.95
2	Chloroform	7.68
3	Ethyl acetate	8.12
4	Acetone	10.80
5	Methanol	16.61
6	Ethanol	7.12
7	70% Ethanol	19.36
8	Aqueous	10.76

Highest extractive value was noted in 70 ethanolic extractive (19.36%)and the lowest extractive value was observed in Petroleum ether (4.95%).

CHAPTER-V

Section-A

This part dealt with the organoleptic evaluation of the *Ipomoea sepiaria* which indicates that the powder material is green in colour, highly characteristic odour and mild astringent taste.

Section-B

Preliminary qualitative phytochemical examination of the powdered leaf and stem were carried out and the results were tabulated in table no-VII. This study indicates the presence of sterols, carbohydrates, proteins, flavonoids, terpenoids, tannins, and saponins.

Preliminary phytochemical screening was also carried out with EEIS. It reveals that ethanolic extract consists of flavonoids, tannins and saponins and the results were tabulated in the table no: VIII

Section-C

This part of the study concerned with preparation of 70% ethanolic extract which was subjected to the estimation of total phenolic content,and flavonoid content. Preliminary phytochemical studies revealed the presence of Phenolic and flavanoids in this extracts. Hence this extract was estimated quantitatively for totalphenolic, and total falvonoid content and the results were shown in the following table.

NAME OF EXTRACT	TOTAL PHENOLIC CONTENT mg/g of extract	TOTAL FLAVONOID CONTENT mg/g of extract
EEIS	206.41±5.970	194.88± 1.54

This study revealed that the ethanolic extract showed significant phenolic and flavonoid content.

Section-D

ISOLATION AND CHARACTERIZATION OF FLAVONOIDS

The dried extract of *Ipomoea Sepiaria* was subjected to column chromatography for the isolation of phytoconstituents. Purification and identification of these compounds were performed by preparative TLC and IR spectrum.

Two mixtures such as mixture-I (pet ether:ethylacetate 80:20) mixture-II (ethylacetate:methanol 80:20) and a single compound (ethylacetate 100%) were obtained as eluates. One compound in mixture-II was identified as quercetin by CO-TLC studies, These two mixtures, mixture-I and mimixture-II were further purified by preparative thinlayer chromatography (PTLC FIG-13.1) and each of these mixture gave two compounds viz compound-I,compound-II,compound-IV and V.

These isolated compounds were given positive reaction for flavonoids, Isolated compound IV in mixture-II was also identified.

Chemical test for flavanoids,CO-TLC studies (Fig-13.2) and IR spectroscopy of compound IV revealed that the compound may be quercetin. Compound-I,II,III and V were identified as flavanoids by chemical tests and IR spectral studies.

CHAPTER-VI

This part of the study deals with the pharmacological evaluation of ethanolic extract of *Ipomoea sepiaria* for antioxidant and acrylamide induced painful neuropathy in rats.

SECTION-A

INVITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *Ipomoea sepiaria* BY DPPH METHODS

H_2O_2 and DPPH antioxidant scavenging activity were carried out for EEIS and the results were tabulated in table no XIII & XIV.

INVITRO ANTIOXIDANT ACTIVITY OF EEIS BY HYDROGENPEROXIDE METHOD

Ethanolic extract was evaluated for H_2O_2 assay which was revealed that the ethanolic extract at 100 µg/ml showed the absorbance of 48.09 % where as the absorbance of standard drug was 86.56%. An increase in absorbance indicates that enhanced reducing potential of ethanolic extract. H_2O_2 assay also indicate the significant antioxidant activity of this extract.

INVITRO ANTIOXIDANT ACTIVITY OF EEIS BY DPPH METHOD

Free radical scavenging activity of ethanolic extract of *Ipomoea sepiaria* (50µg/ml) was found to be 43.50 % when compared to the radical scavenging activity of standard drug vitamin-C which was found to be 96.94 %. IC_{50} value of the ethanolic extract was 99.80µg/ml and for standard drug (vit-C) 17.96255 µg/ml. This study indicate antioxidant activity of this extract.

INVITRO ANTIOXIDANT ACTIVITY OF ISOLATED COMPOUNDS OF *Ipomoea sepiaria* BY DPPH METHOD

Isolated compounds obtained by column chromatography such as mixture-I, mixture-II and single compound were evaluated for antioxidant activity by DPPH radical scavenging method. Mixture-II exhibited potent antioxidant activity (64.56µg/ml)when compare to mixture-I (170.81 µg/ml) and single compound (135.10 µg/ml). Neuroprotective activity of flavonoids against toxin induced neuropathy was attributed due to their antioxidant potential.[101] This study revealed that mixture-II exhibited significant antioxidant activity than mixture-I and single compound.Hence mixture-II was administered to the rats (5mg/kg body weight) in order to evaluate its efficacy against acrylamide induced painful neuropathy.

Section-B

Effect of EEIS on Paw cold allodynia

Acrylamide induced toxicity of Sciatic nerve resulted significant development of non-noxious cold chemical allodynia, noted by decrease in hind paw withdrawal threshold after 6^{th} day of acrylamide intoxication as compared to normal control group. Acrylamide induced, decrease in nociceptive threshold for cold allodynia was improved by the administration of EEIS (100&200mg/kg, p.o) and Isolated mixture compound-II (5mg/kg,p.o) . Treatment of pregabalin also produced similar effects. However statistically significant attenuation was recorded in all the groups treated with EEIS (100 & 200mg/kg,p.o, $P < 0.05$) and Isolated mixture compound-II (5mg/kg,p.o $P < 0.05$).

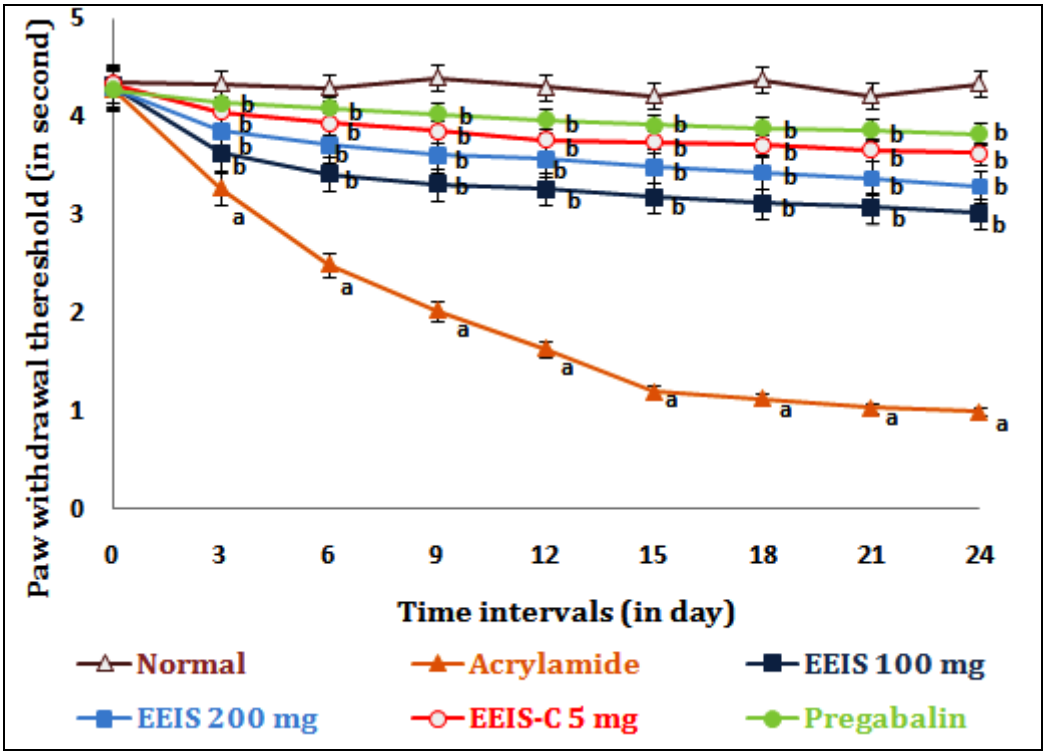
Effect of EEIS on paw heat hyperalgesic test

Acrylamide induced toxicity of Sciatic nerve resulted significant development of noxious thermal hyperalgesia noted by decrease in hind paw withdrawal threshold after 6^{th} day of acrylamide intoxication as compared to normal control group. Acrylamide induced, decrease in nociceptive threshold for thermal hyperalgesia was improved by the administration of EEIS (100&200mg/kg, p.o) and Isolated mixture compound-II (5mg/kg,p.o) . Treatment of pregabalin also produced similar effects. However statistically significant attenuation was recorded in all the groups treated with EEIS (100 & 200mg/kg, p.o, $P < 0.05$) and Isolated mixture compound-II (5mg/kg, p.o $P < 0.05$).

Tail immersion test

Acrylamide induced toxicity of Sciatic nerve resulted significant development of noxious thermal hyperalgesia noted by decrease in tail withdrawal threshold after 6^{th} day of acrylamide intoxication as compared to normal control group. Acrylamide induced, decrease in nociceptive threshold for thermal hyperalgesia was improved by the administration of EEIS (100&200mg/kg, p.o) and Isolated mixture compound-II (5mg/kg,p.o).Treatment of pregabalin also produced similar effects. However statistically significant attenuation was recorded in all the groups treated with both EEIS (100 & 200mg/kg, p.o) and Isolated mixture compound-II (5mg/kg,p.o $P < 0.05$).

Fig.30. Effect of Ethanolic extract of *Ipomoea sepiaria* by paw cold allodynia



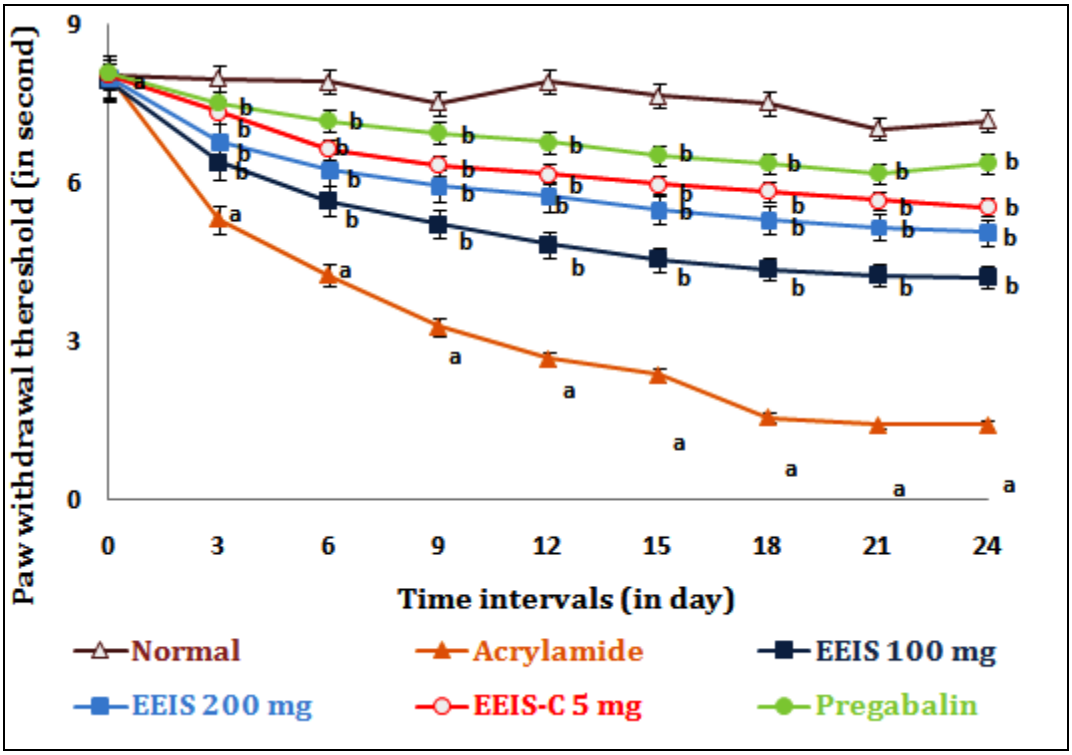
Data were expressed as mean ± SD (n=6).

EEIS,(Ethanolic extract of ipomoea sepiaria); EEIS-C,(Ethanolic extract of ipomoea sepiaria isolated compound)

[a]*P < 0.05* Vs. normal group.

[b]*P < 0.05* Vs. acrylamide control group.

Fig.31. Effect of Ethanolic extract of *Ipomoea sepiaria* on hot plate test



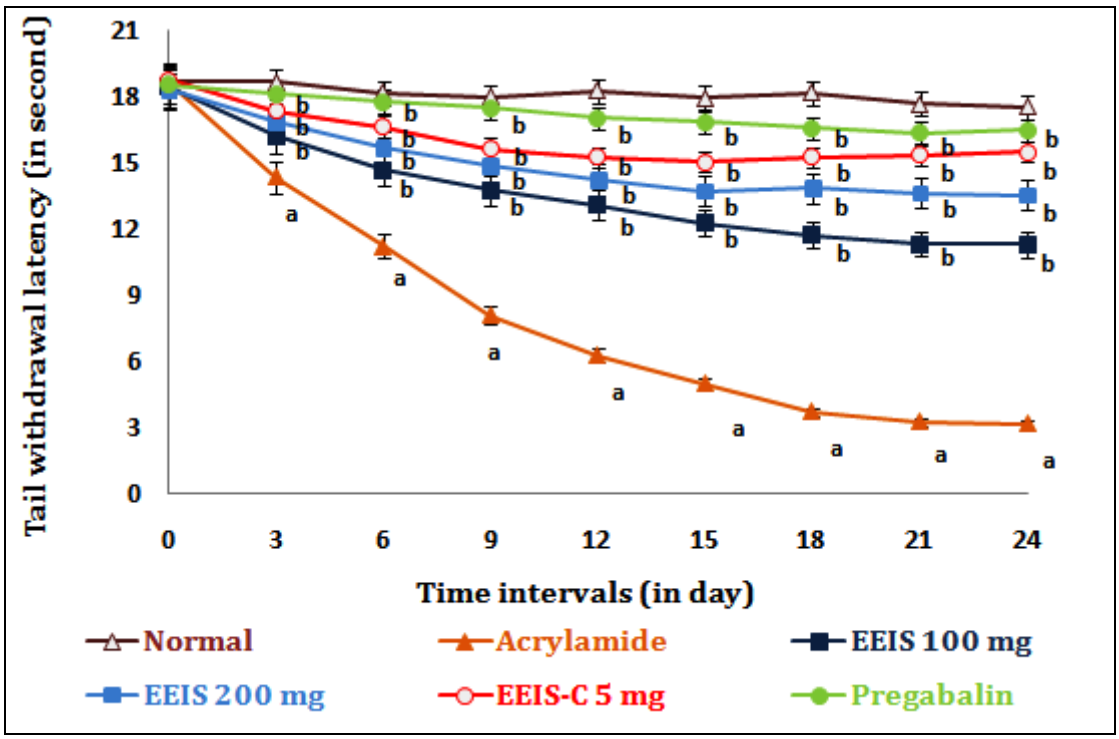
Data were expressed as mean ± SD (n=6).

EEIS,(Ethanolic extract of ipomoea sepiaria); EEIS-C,(Ethanolic extract of ipomoea sepiaria isolated compound);

[a]$P < 0.05$ Vs. normal group.

[b]$P < 0.05$ Vs. acrylamide control group.

Fig.32. Effect of Ethanolic extract of *Ipomoea sepiaria* on tail immersion test



Data were expressed as mean ± SD (n=6).

EEIS,(Ethanolic extract of ipomoea sepiaria); EEIS-C,(Ethanolic extract of ipomoea sepiaria isolated compound)

[a]$P < 0.05$ Vs. normal group.

[b]$P < 0.05$ Vs. acrylamide control group.

Effect of EEIS on oxidative stress markers

Acrlyamide induced sciatic nerve intoxication resulted in significant rise in TBARS,(56.41nmol,$p<0.05$) total calcium level (16.87ppm,$p<0.05$) and decrease in the level of reduced glutathione (12.64 µg/mg,$p<0.05$) after the 24^{th} day of drug administration as compared to normal control group. Administration of EEIS and isolated mixture compound attenuated acrylamide induced rise in sciatic nerve tissue level of TBARS and total calcium, and the decreased level of reduced glutathione. Administration of EEIS (100 mg/kg., p.o) resulted attenuation of TBARS (29.2 nmol, $p<0.05$) and total calcium (7.16 ppm, $p<0.05$) and rise in the level of reduced glutathione (31.78µg/mg,$p<0.05$). EEIS treatment (200mg/kg., p.o) resulted the reduction of TBARS (21.2 nmol, $p<0.05$) and total calcium (5.48 ppm, $p<0.05$) and increased in the level of reduced glutathione (54.63 sµg/mg,$p<0.05$). Administration of isolated mixture compound-II (5 mg/kg.p.o) was found to be decreased TBARS (20.55 nmol, $p<0.05$) and total calcium (3.64 ppm, $p<0.05$) and elevated the level of reduced glutathione (59.51µg/mg,$p<0.05$).

Effect of histopathological changes

Acrylamide induced toxicity of sciatic nerve resulted in significant histopathological changes assessed in cross section of the sciatic nerve. In the cross section, acrylamide induced axonal degeneration and dearrangement of nerve fibres were observed. Administration of EEIS and isolated mixture compound-II (100mg,200mg & 5 mg/kg, p.o) significantly attenuated acrylamide induced axonal degeneration and dearrangement of nerve fibres.

Discussion

In the present study, EEIS and isolated mixture compound-II treatment normalize acrylamide induced sciatic nerve intoxication mediated behavioural (heat hyperalgesia [paw & tail], cold allodynia) and biochemical abnormalities (TBARS, total calcium & reduced glutathione). Acrylamide toxicity causes the "dying back" neuropathy in rats (distal axonopathy). Acrylamide is a neurotoxic agent, is used in industries which involved in the manufacture of dye and fibre, polymer production, gel electrophoresis and water treatment. Acrylamide exposure leads to central-peripheral distal axonopathy in peripheral nervous system.

The underlying mechanism for the cause of acrylamide intoxicated neuropathy is still not yet understood. Recently oxidative stress has been demonstrated to be one of the key mechanism in many chemical- induced cell injuries. Oxidative stress in the cells or tissues refers to enhanced generation of ROS and/or depletion of antioxidant defense system, causing an imbalance between pro oxidants and antioxidants, potentially leading to damage.

If the intracellular reactive oxygen species level increases may lead to damage the mitochondria, lipid peroxidation, elevated cytokine production and cell death. ROS generation in tissues is effectively scavenged by enzymatic antioxidant system, (such as SOD, GSH-Px, CAT, and GR) and non enzymatic antioxidants (such as GSH, Vitamin A, C, and E). This present study revealed that TBARS level of the acrylamide treated group was found to be elevated and it was found to be decreased significantly in EEIS (100mg/kg 200mg/kg; $P < 0.05$) and isolated mixture compound (5mg/kg; $P < 0.05$) treated animals.

These findings indicate that acrylamide induced lipid peroxidation denoted by elevated TBARS level was markedly controlled by EEIS treatment at 100mg,200mg & isolated mixture compound 5mg/kg, p.o. Documented studies revealed that ROS can attack the polyunsaturated fatty acid in the biomembrane to initiate the free radical chain reaction.

Acrylamide treatment significantly reduces the non enzymatic endogenous antioxidant GSH level and the EEIS treatment 100mg,200mg & isolated mixture compound 5mg/kg, p.o significantly increases the level of GSH. The above data indicate that EEIS treatment resulted elevation of endogenous antioxidant GSH. GSH which can effectively scavenge free radicals directly and indirectly which is the major non enzymatic antioxidant in cells.

GSH plays an important role in antioxidant defense, nutrient metabolism, and regulation of cellular events. GSH deficiency contributes to oxidative stress, which takes effect in the pathogenesis of many diseases, e.g., Alzheimer's disease and neuropathy.

Documented studies showed that conjugation with glutathione (GSH) is a mechanism for the detoxification of acrylamide. Glycidamide, an active neurotoxic metabolite of acrylamide, can also conjugate with GSH. The acrylamide induced depletion of GSH may make the nerve tissue more sensitive to the oxidative stress which is significantly reversed by EEIS treatment.

Acrylamide treatment increases total calcium level and treatment of EEIS at 100mg/kg, 200mg/kg and isolated mixture compound 5mg/kg, significantly reduces the total calcium level. The noted decrease in calcium level with EEIS may be attributed to its antioxidant effect, as free radicals are well reported to increase

calcium ions. However the possible action of EEIS on decrease in calcium level may not also be ruled out. Moreover increase in calcium ions is also associated with increase in oxidative stress. So the noted antioxidant effects of EEIS may also be ascribed secondary to decrease in calcium ions. Pregabalin treatment also increases GSH level, decreases TBARS and total calcium level. Oxidative stress may impair axonal membrane which was attenuated by EEIS treatment. EEIS showed significant antioxidant activity in ***invitro*** assays like DPPH and H_2O_2 . Neuroprotective effect of EEIS against acrylamide induced neuropathy may be due to the antioxidant potential of this extract, which has been proved by both ***invivo*** and ***invitro*** antioxidant assays. Antioxidant property of EEIS may be due to the presence of tannins and flavonoids.

Quercetin has already been reported for its neuroprotective effect against alcohol induced neuropathy by attenuating thermal hyperalgesia and also through modulation of membrane bound inorganic phosphate enzyme and inhibition of release of oxides,Inflammation mediators such as MDA (Melodialdehyde), MPO (Myeloperoxidase), and nitricoxide[102] .

Quercetin has proven to protect against the development of diabetic neuropathy by inhibition of lipid peroxidation and restoration of antioxidant enzyme in diabetic rats, Thus reverse the oxidative stress induced changes in nerve physiology of diabetic rats are reported earlier. [103]

Phytochemical studies revealed that Compound mixture-II consist of two compounds and one of the identified constituent may be quercetin, Administration of compound mixture –II to the acrylamide intoxicated rats resulted attenuation of behavioural parameters such as paw and tail heat hyperalgesia ($P< 0.05$)and cold allodynia ($P<0.05$)and biochemical parameters such as TBARS($P<0.05$) total

calcium($P<0.05$) and reduced GSH($P<0.05$) as well as histopathological changes. Neuroprotective effect of compound mixture-II may be due to the presence of quercetin in this mixture. Isolated compound mixture and EEIS possess therapeutic potential on acrylamide induced biochemical and histopathological changes in rats.These ameliorative effect may be attributed due to the anti oxidative and neuroprotective potential of EEIS and isolated compound mixture-II and quercetin may be responsible for these activities.

IN VITRO ANTI CANCER ACTIVITY

Ethanolic extract of *Ipomoea sepiaria* was effective against invitro liver cancer cell lines of HepG2 cells at 500µg and the percentage cell inhibition was found to be 90.37% Quercetin has been reported for anti cancer activity. Hence the anti cancer activity of EEIS may be due to the presence of quercetin.[104]

CHAPTER-VIII

CONCLUSION

Many unknown plants, lesser known plants are used in folk and tribal medicine practices as a source of medicine. The medicinal values of these plants are not brought into the light of scientific world. One such plant is *Ipomoea sepiaria*.keeping in this view an attempt was made to bring light to the commonly available plant *Ipomoea sepiaria*.

This dissertation work entitled Pharmacognostical, phytochemical and the Pharmacological studies which have been dealt in this study.

Preliminary phytochemical screening of the Leaf and stem of this plant confirms that the presence of sterols, terpenoids, carbohydrates, proteins, flavonoids, tannins and saponins.

Estimation of total phenolics and flavonoids were determined for the ethanolic extract of this plant.

Phytoconstituents like flavonoids were isolated by column chromatography, Mixture-I,mixture-II and a single compound were isolated, which gave positive reaction for flavonoids.Co- TLC Studies With quercetin revealed that one among the compound of mixture-II may be quercetin, Mixture-I and mixture-II were purified by preparative thinlayer chromatography(PTLC).Five compounds were isolated and compound IV was identified and this may be quercetin, which was evidenced by CO-TLC with standard quercetin. TLC,chemical test and IR spectral studies revealed that all the isolated compounds were flavanoids and compound IV may be quercetin.

Antioxidant potential of isolated compounds such as mixture-I,mixture-II and single compound were determined by DPPH method,mixture-II has significant radical scavenging property than mixture-I and single compound.Hence it was evaluated for invivo acrylamide induced painful neuropathy in rats.

PHARMACOLOGICAL SCREENING CONFIRMS,

Poly phenol secondary metabolites exhibited potent antioxidant activity. Quantitative estimation of EEIS showed significant total phenolic and flavanoid content.

EEIS exhibited potent antioxidant activity by invitro assays like DPPH and H2O2 scavenging activity.It was further corroborated by the elevation of non enzymic endogenous antioxidant GSH and decreased in TBARS which is a marker compound for LPO (lipid peroxides) and reduced calcium ion levels in the nerve tissue homogenate of the EEIS treated acrylamide intoxicated animals.

It was concluded that EEIS showed significant antioxidant activity in both invitro and invivo antioxidant models.

EEIS treatment at 100mg/kg, 200mg/kg significantly normalizes the acrylamide intoxicated behavioural, biochemical, and histopathological changes and these effects may be due to the presence of antioxidant phytoconstituents like flavonoids.

The isolated mixture compound-II was given at 5 mg/kg to the animals which showed significant neuroprotective effect for acrylamide induced painful neuropathy and this may be due to the antioxidant potential of quercetin and other flavonoid compounds.[101-103]

EEIS at 500µg showed significant invitro anticancer activity against HepG2 liver tumour cell lines. Quercetin like flavonoids have been reported for anti cancer activity[104].Hence it is suggested that the active principle responsible for treating painful neuropathy and liver cancer may be isolated from *Ipomoea sepiaria* and evaluated for further studies.

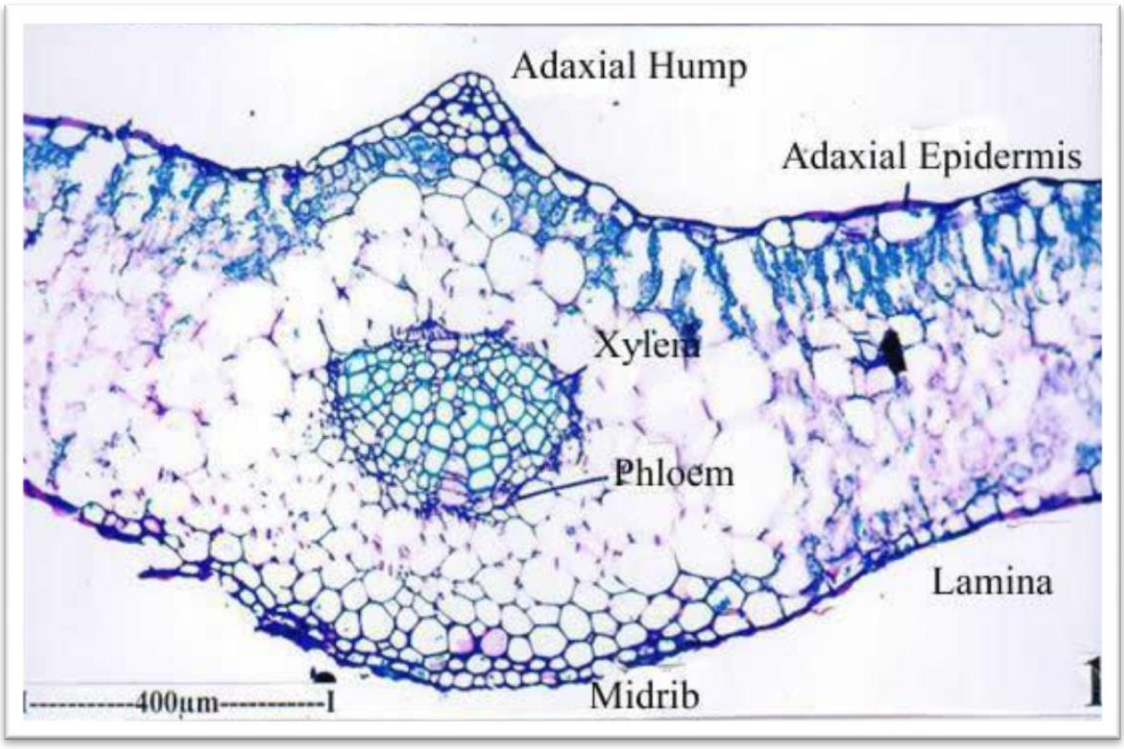
FIG- 1 HABIT AND HABITAT OF *Ipomoea sepiaria*

FIG - 2 DARSAL VIEW OF *Ipomoea sepiaria*

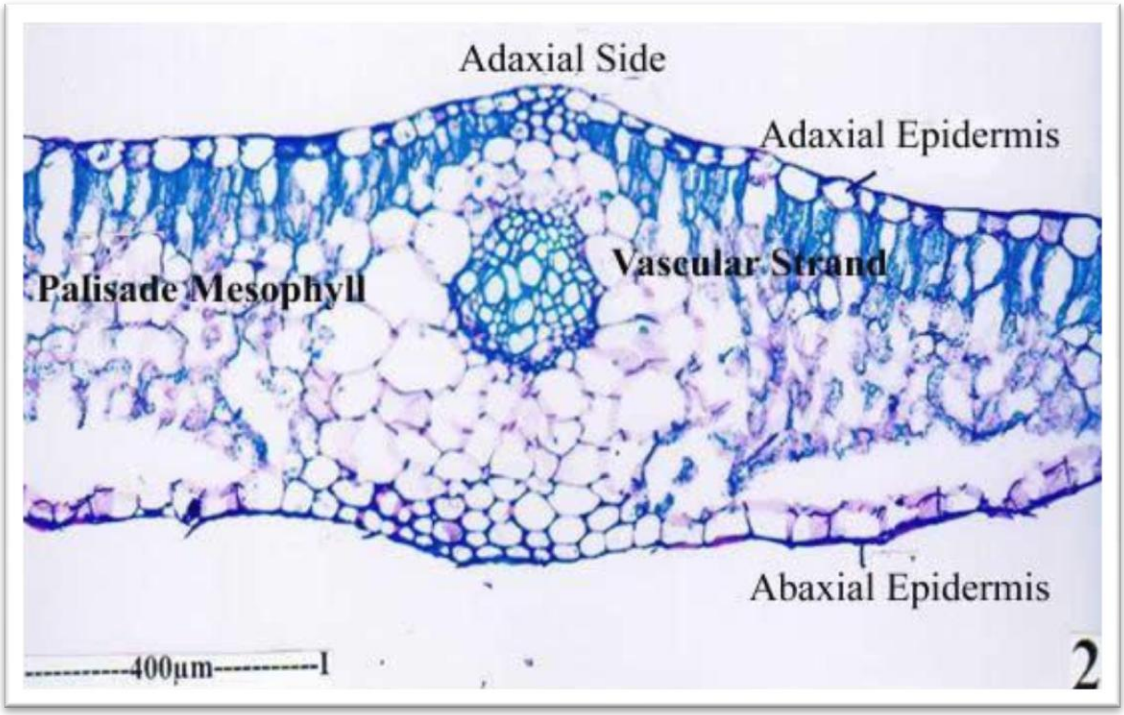
FIG - 3 VENTRAL VIEW *Ipomoea sepiaria*

FIG - 4 FLOWER AND STEM OF *Ipomoea sepiaria*

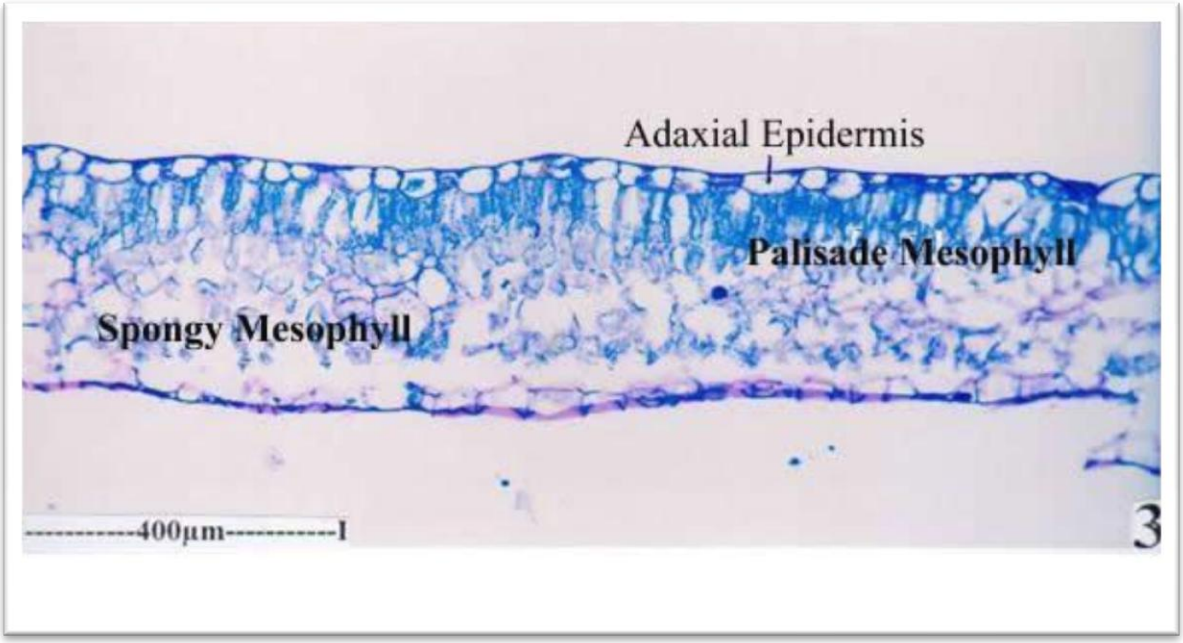
T.S OF LEAF THROUGH MIDRIB (FIG.5.1)



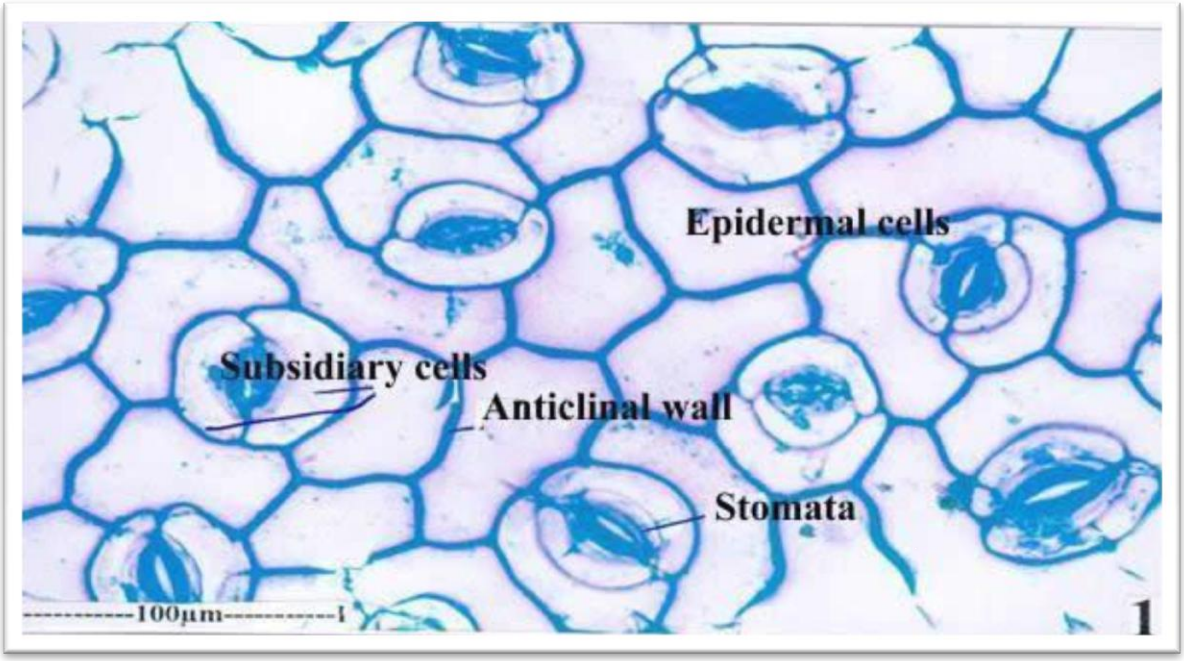
T.S OF LEAF THROUGH LATERAL VIEW (FIG.5.2)



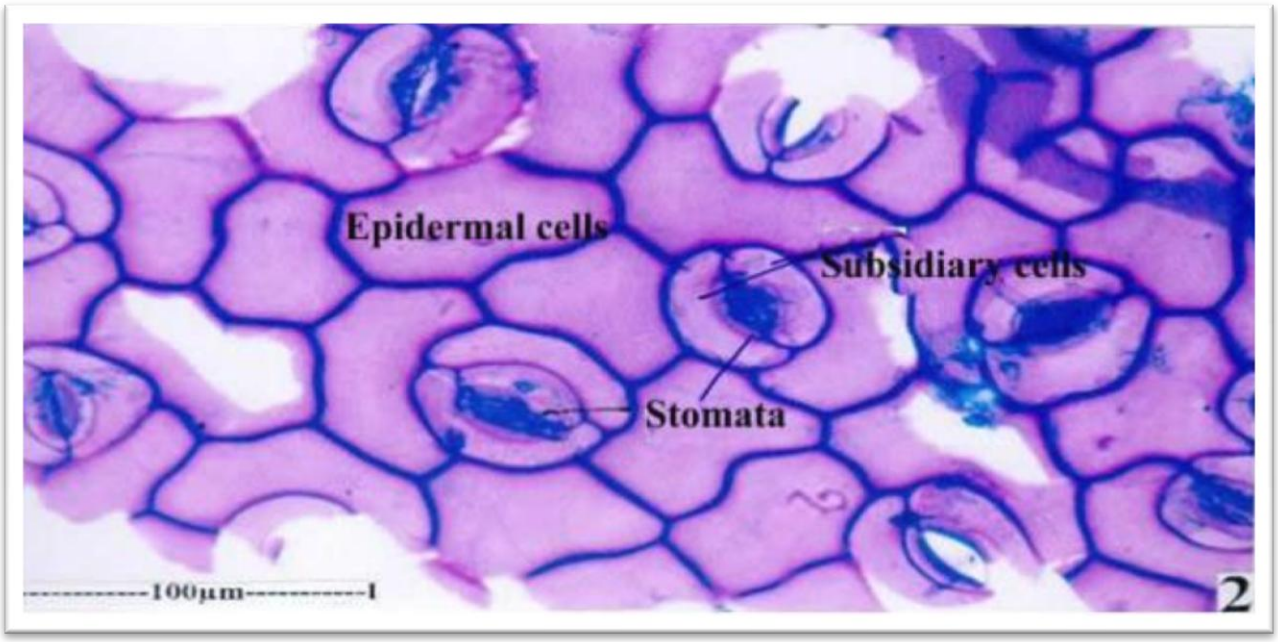
T.S OF LAMINA FIG.5.3



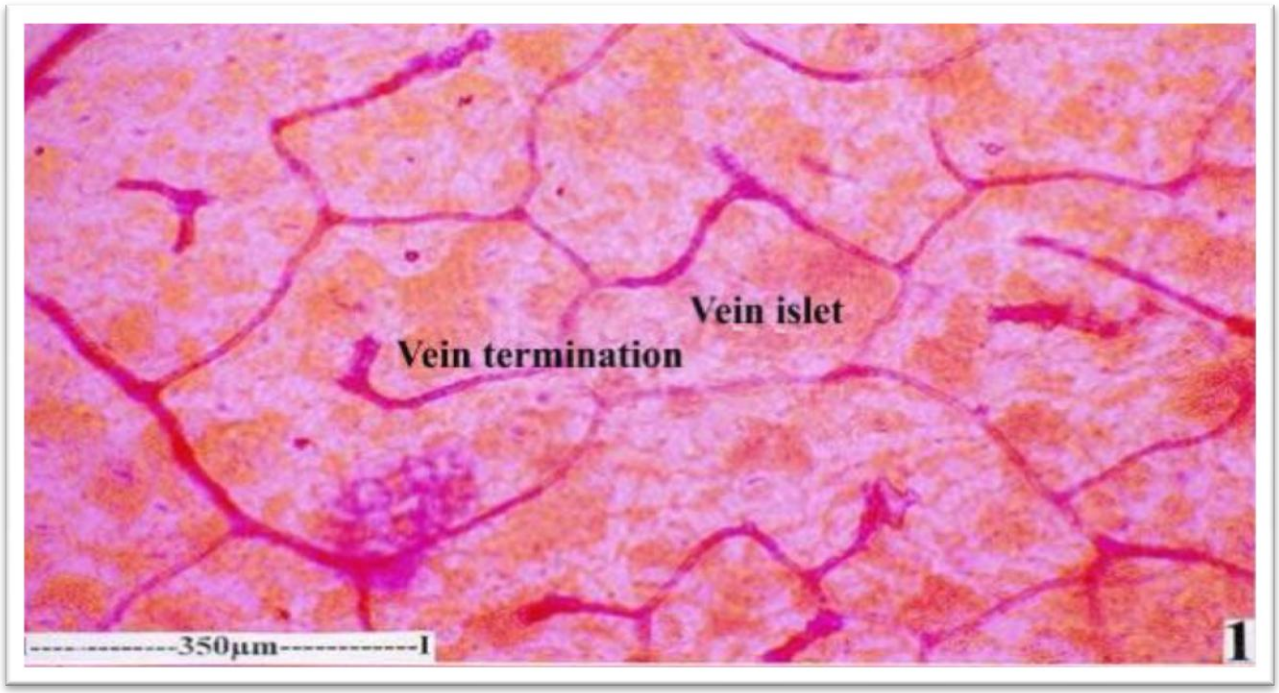
PARADERMAL SECTION S OF THE LAMINA FIG 6.1



SHOWING EPIDERMAL CELLS AND PARACYTIC STOMATA FIG.6.2

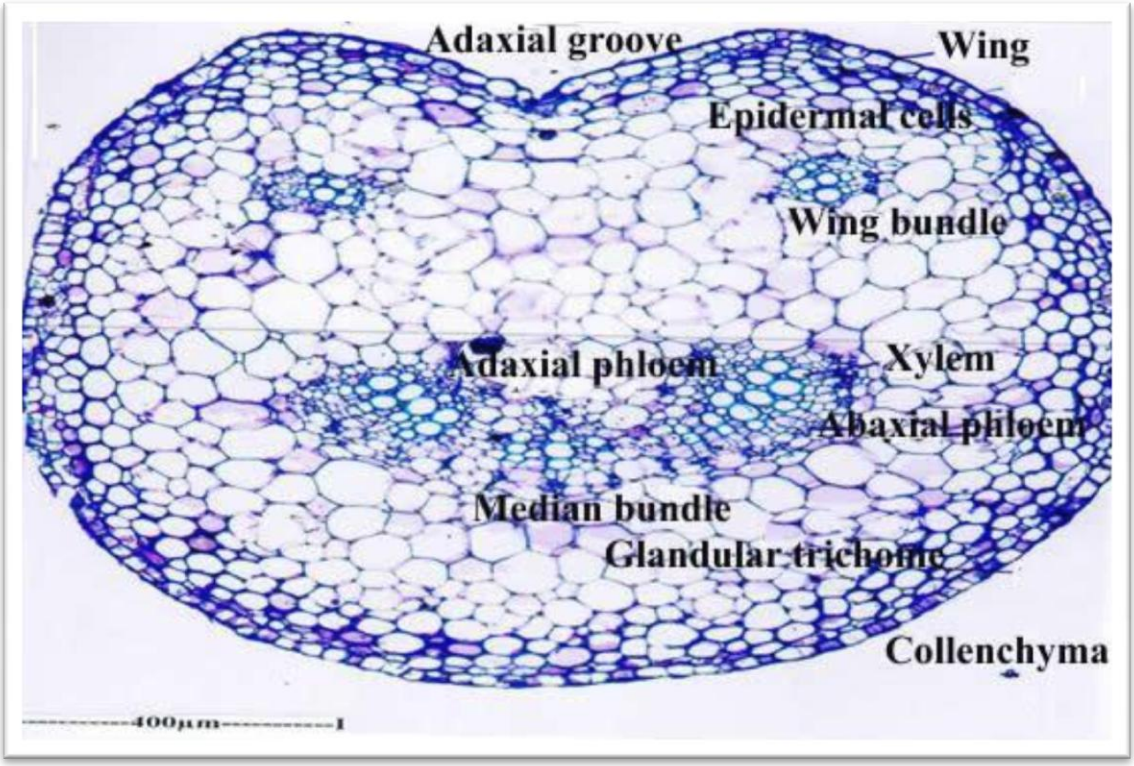


LAMINA CLEARED SHOWING THE VENATION PATTERN FIG.7.1

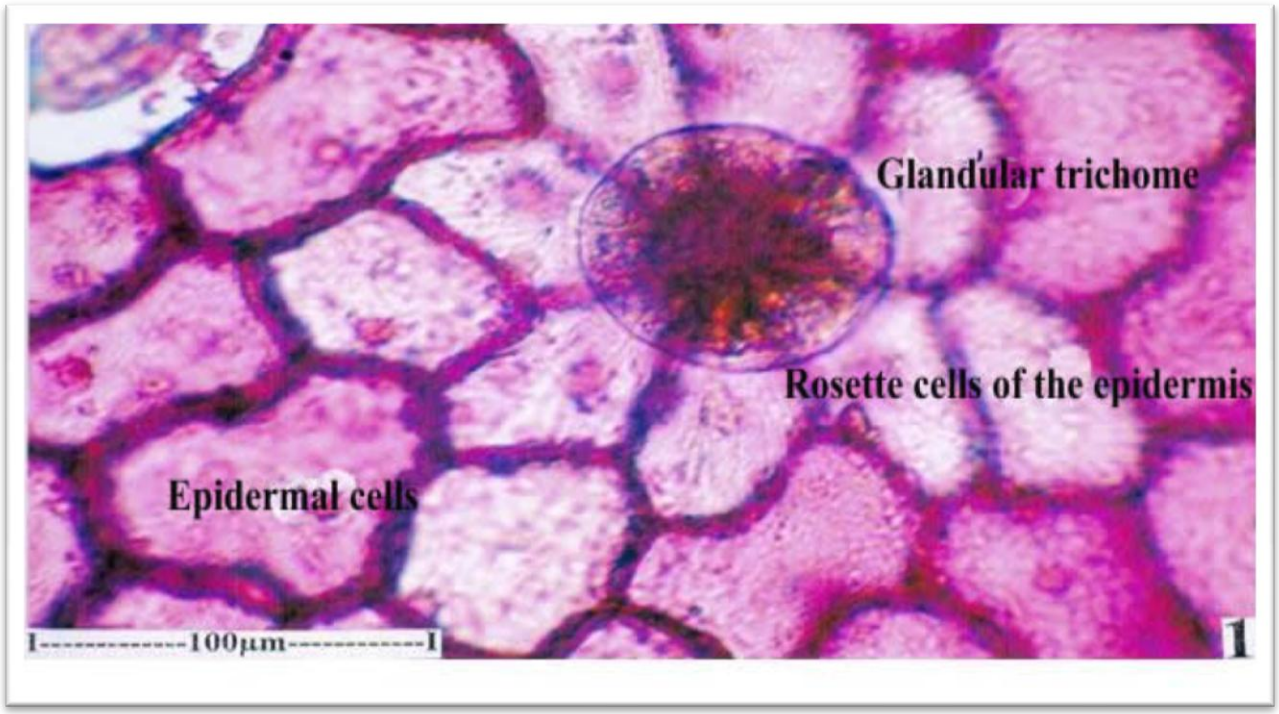


VEIN TERMINATION WITH TERMINAL TRACHEID FIG.7.2

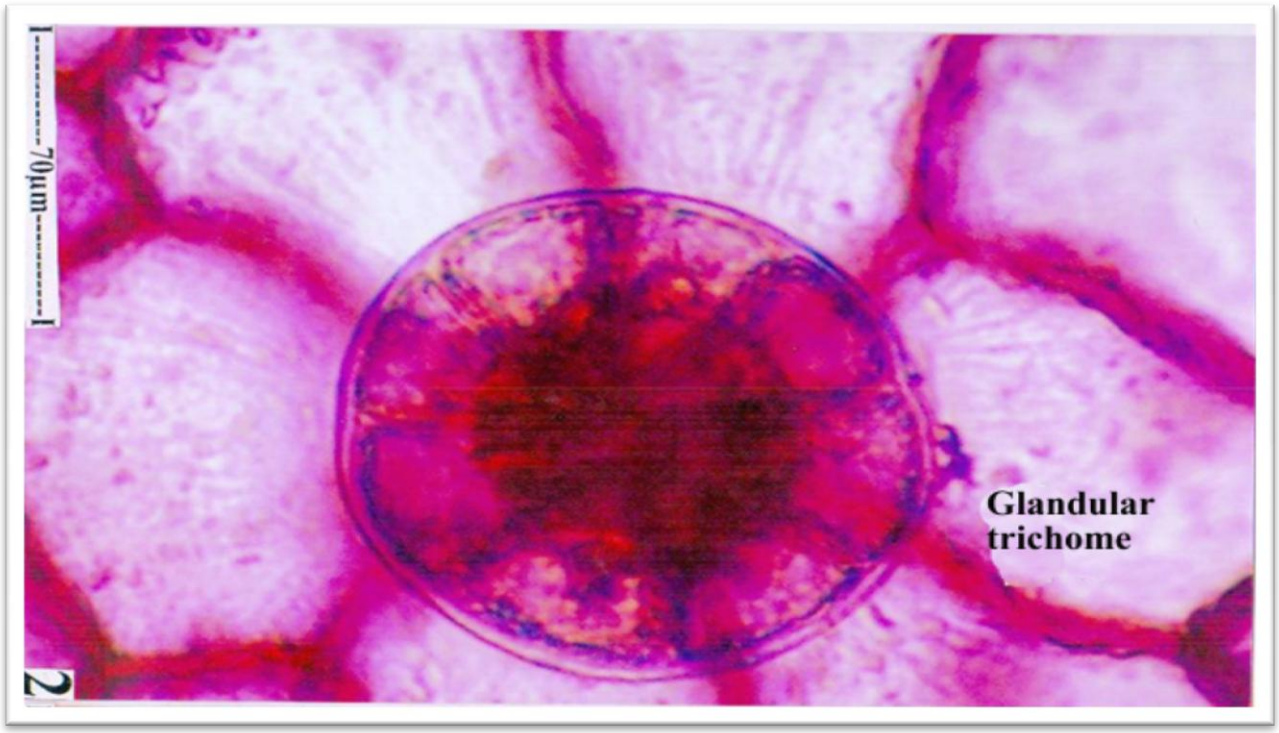
T.S OF PETIOLE-ENTIRE VIEW FIG.8



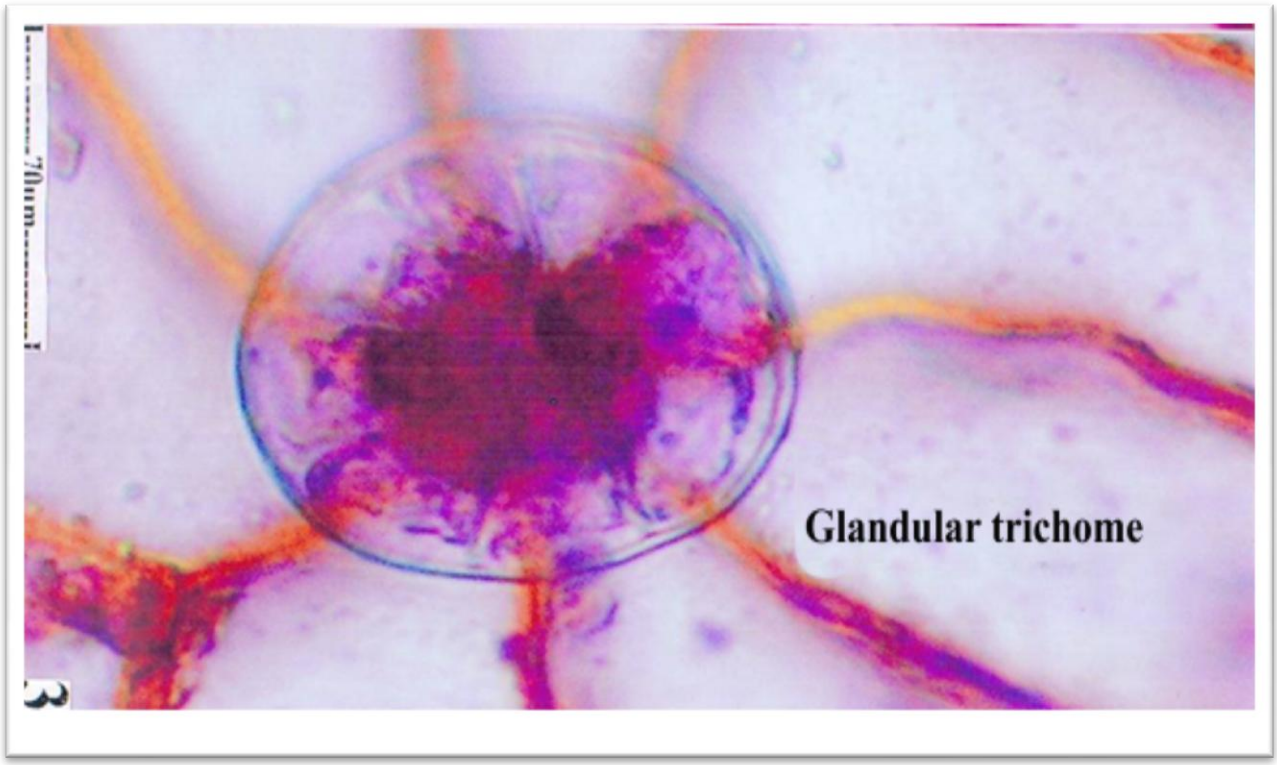
GLANDULAR TRICHOME OF THE ADAXIAL VIEW FIG - 9.1



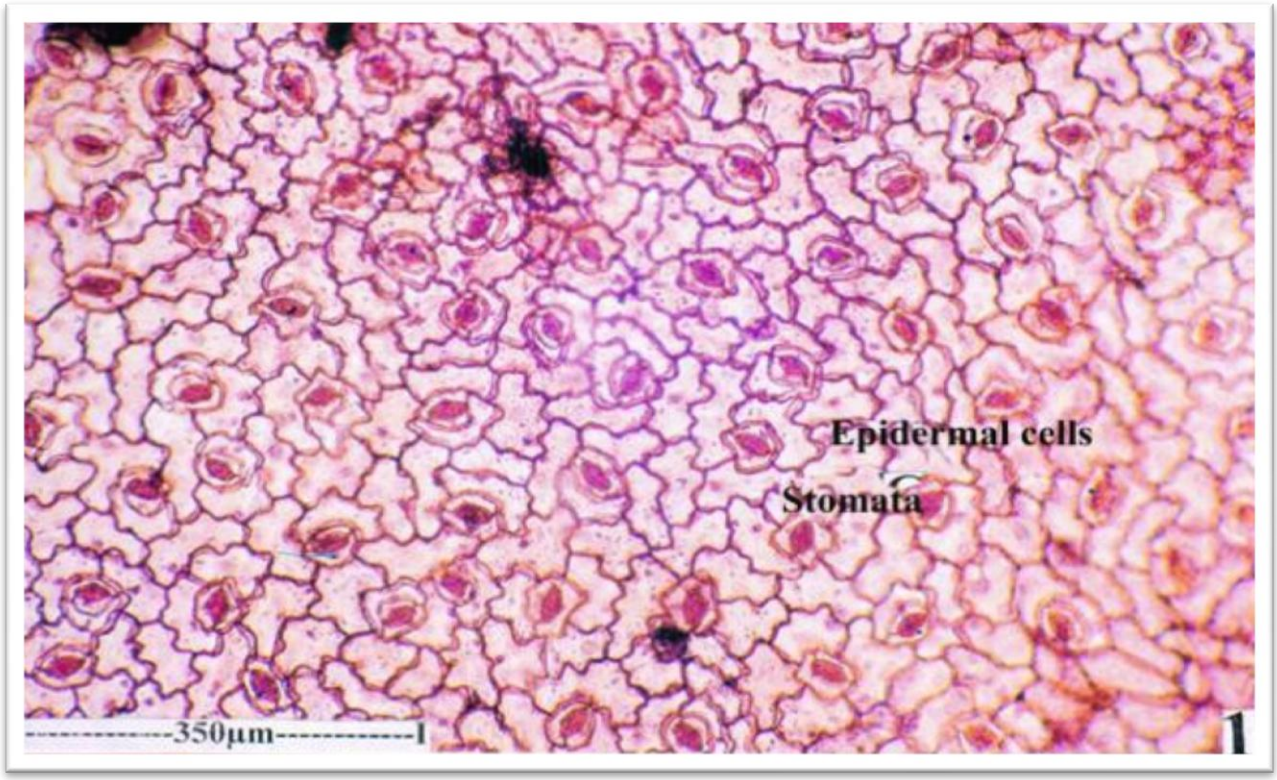
EPIDERMIS AS SEEN IN AERIAL VIEW FIG - 9.2



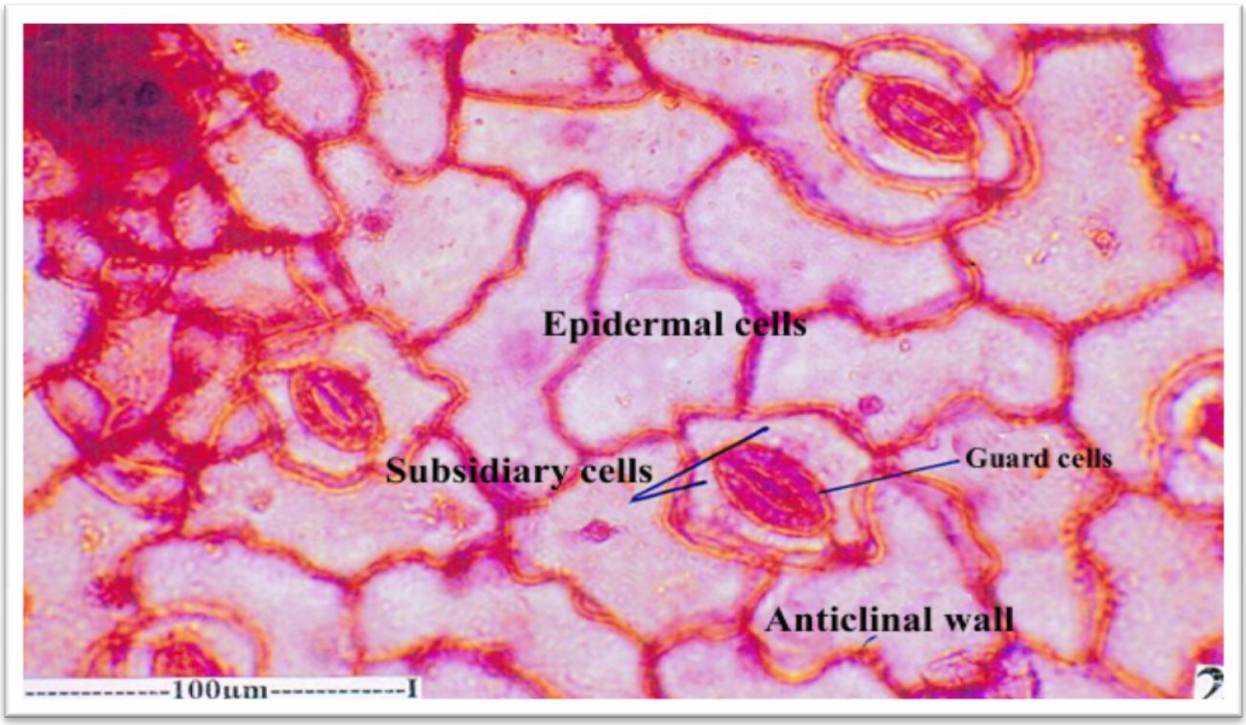
GLANDULAR TRICHOME ON THE ABAXIAL EPIDERMIS FIG -9.3



STOMATA AND EPIDERMAL TISSUE OF THE ABAXIAL EPIDERMIS FIG - 10.1

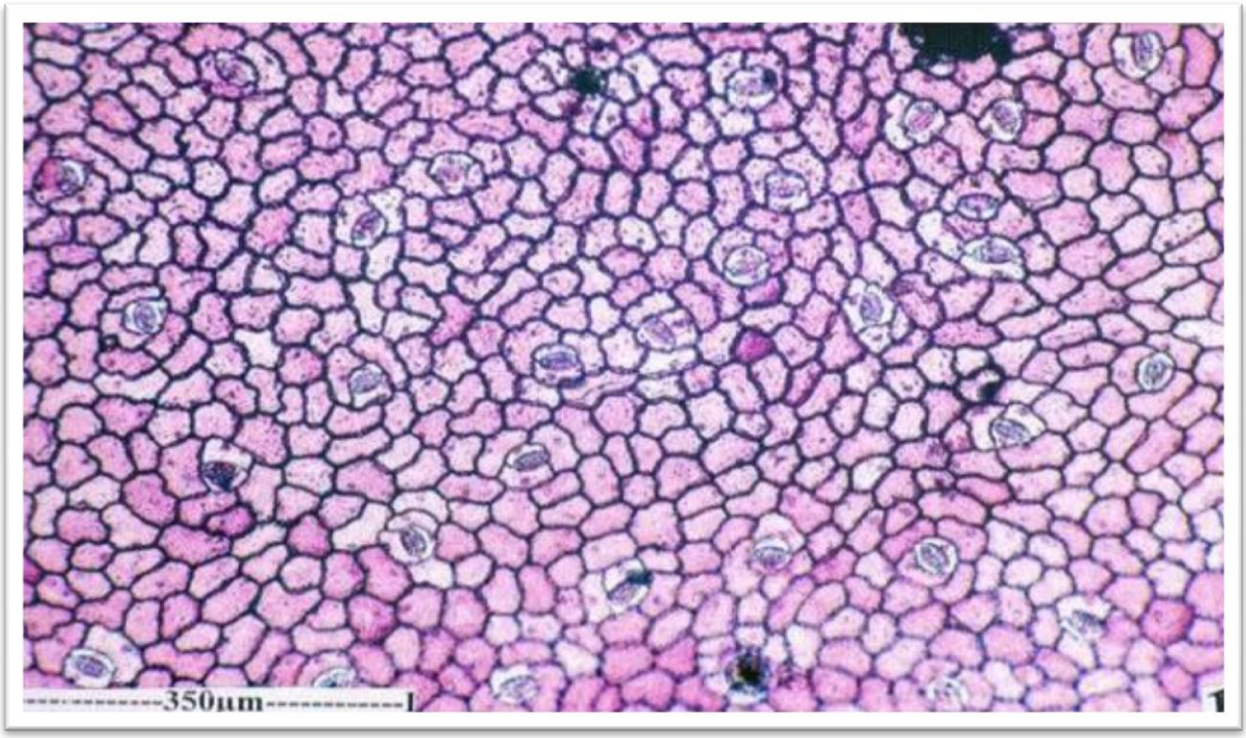


A FEW STOMATA ENLARGED FIG - 10.2

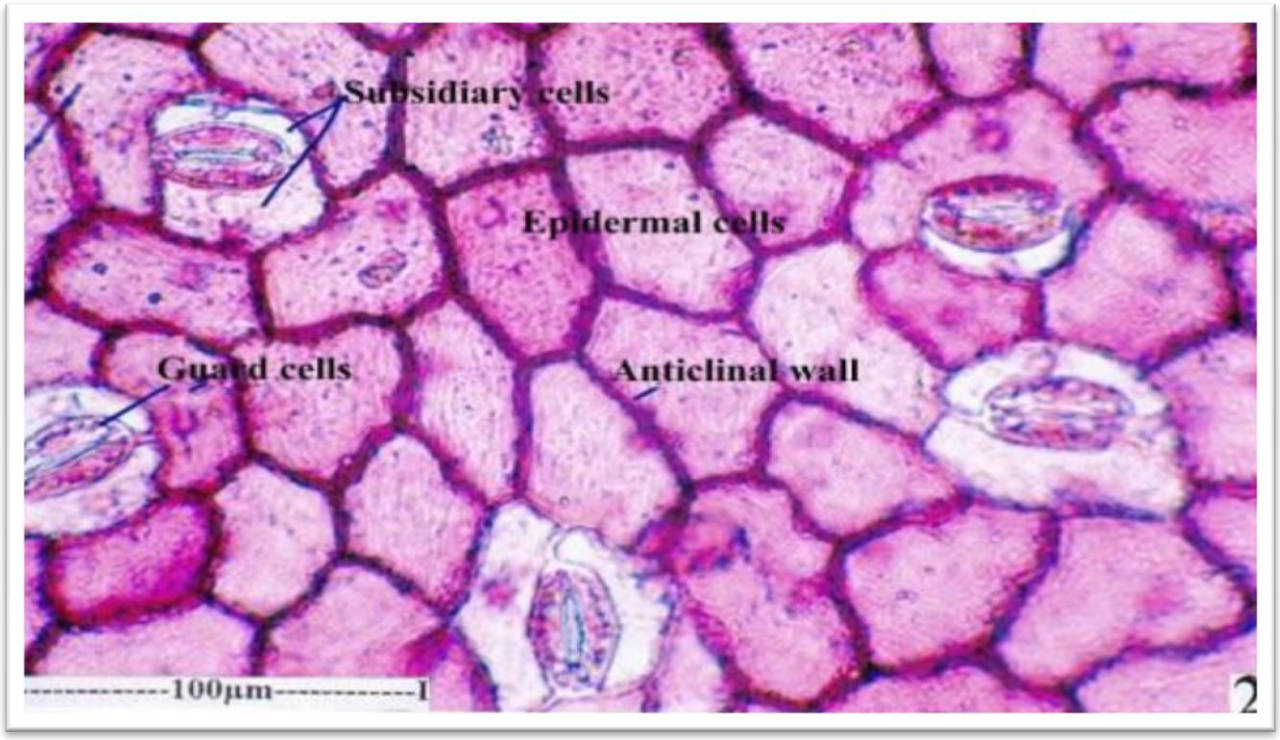


EPIDERMAL TISSUE AND STOMATA OF THE ADAXIAL SIDE

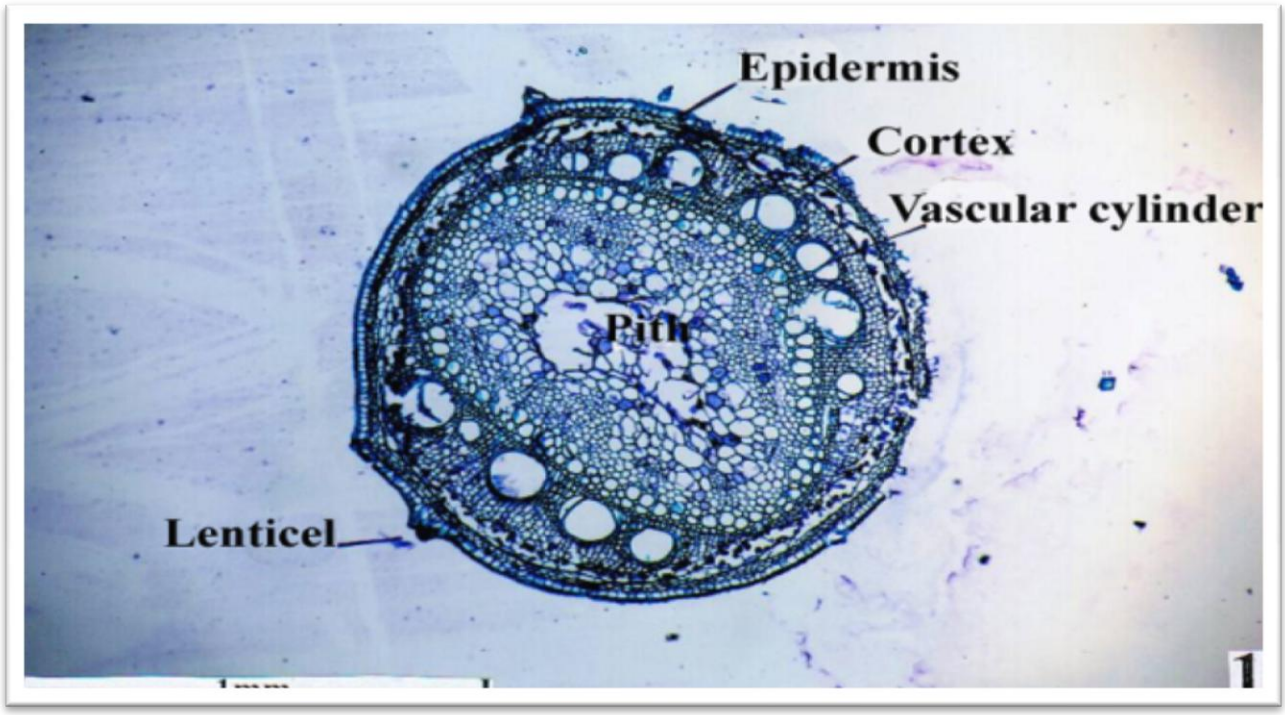
FIG-11.1



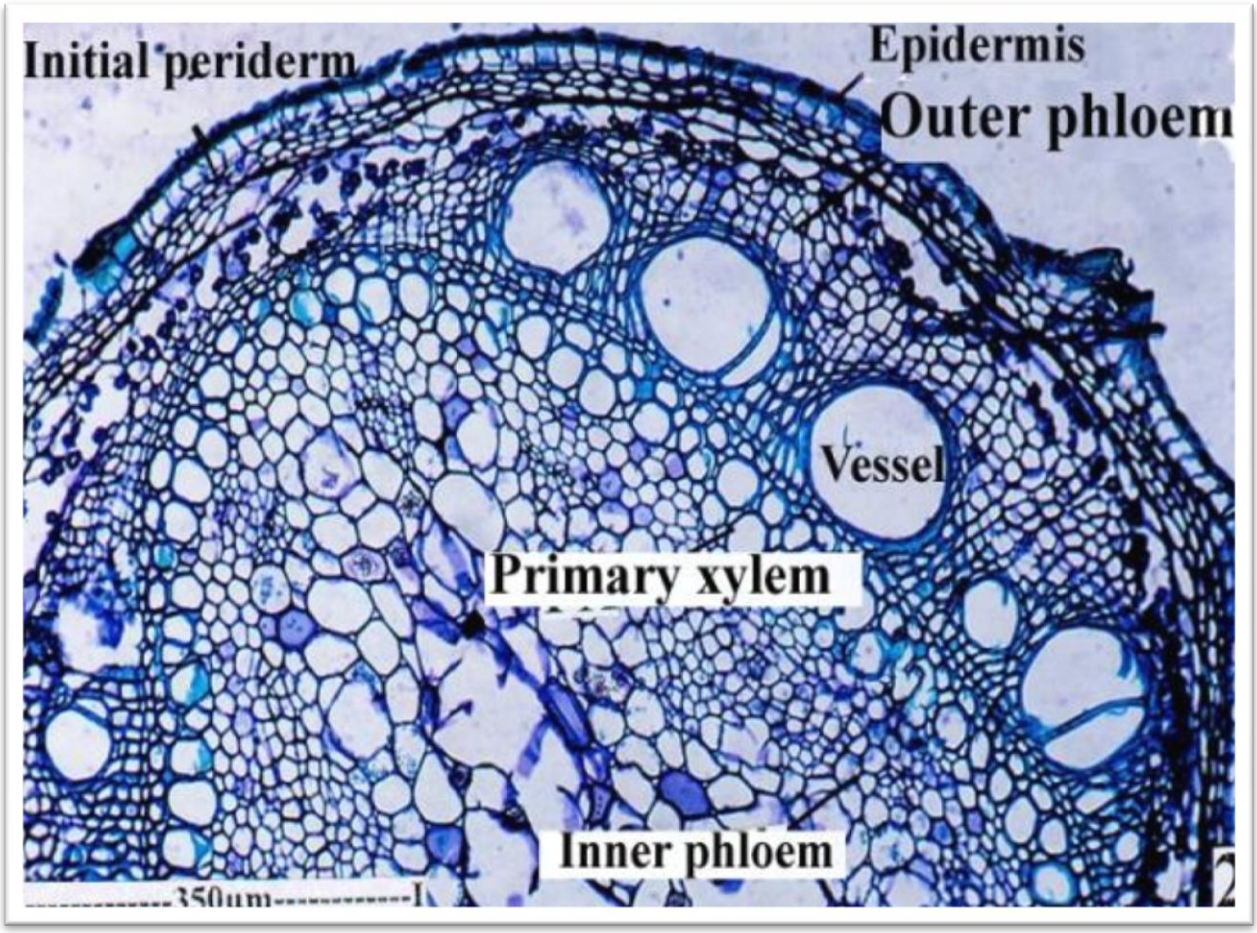
A PORTION OF THE ABOVE ENLARGED FIG - 11.2



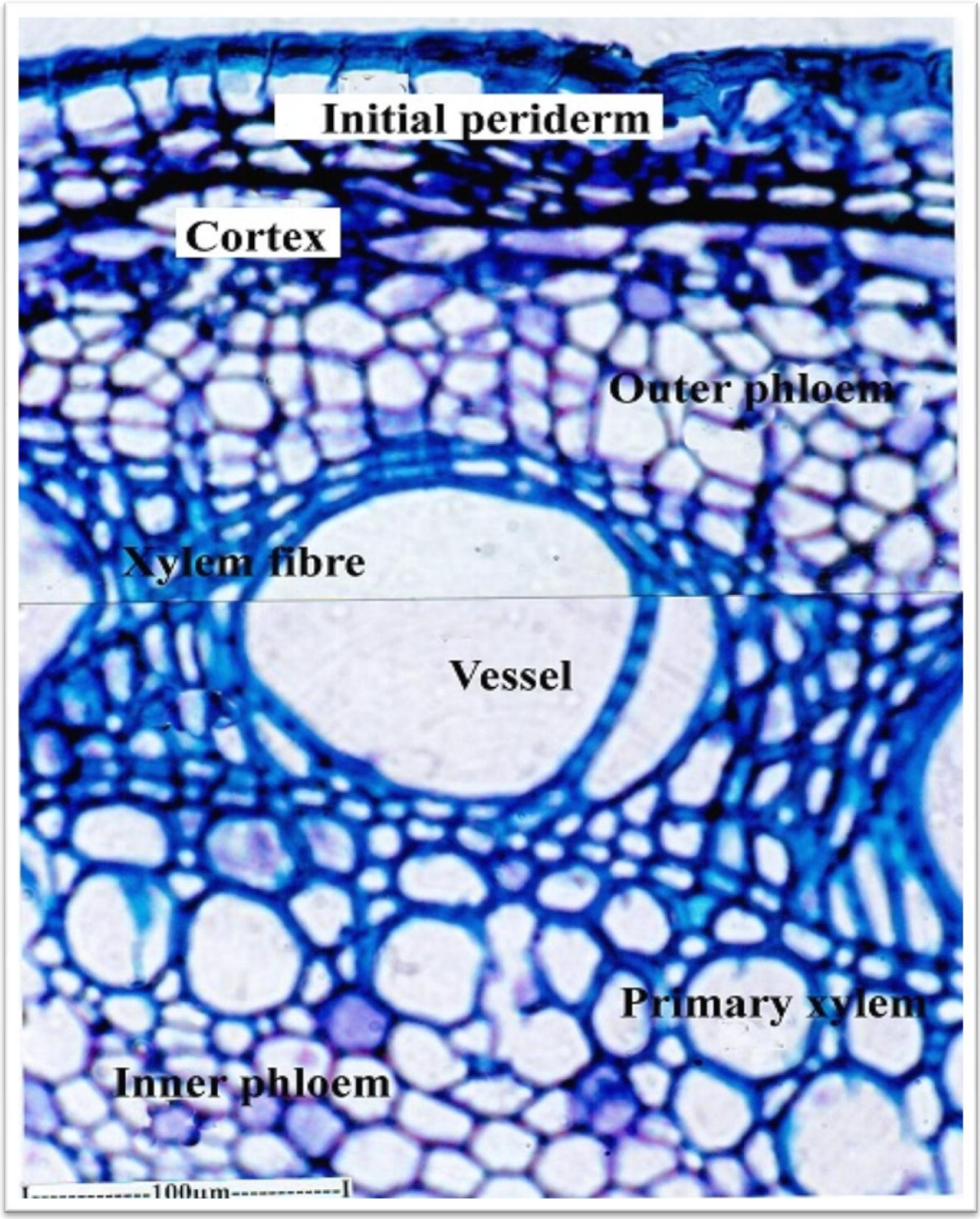
T.S OF STEM INITIAL VIEW FIG -12.1



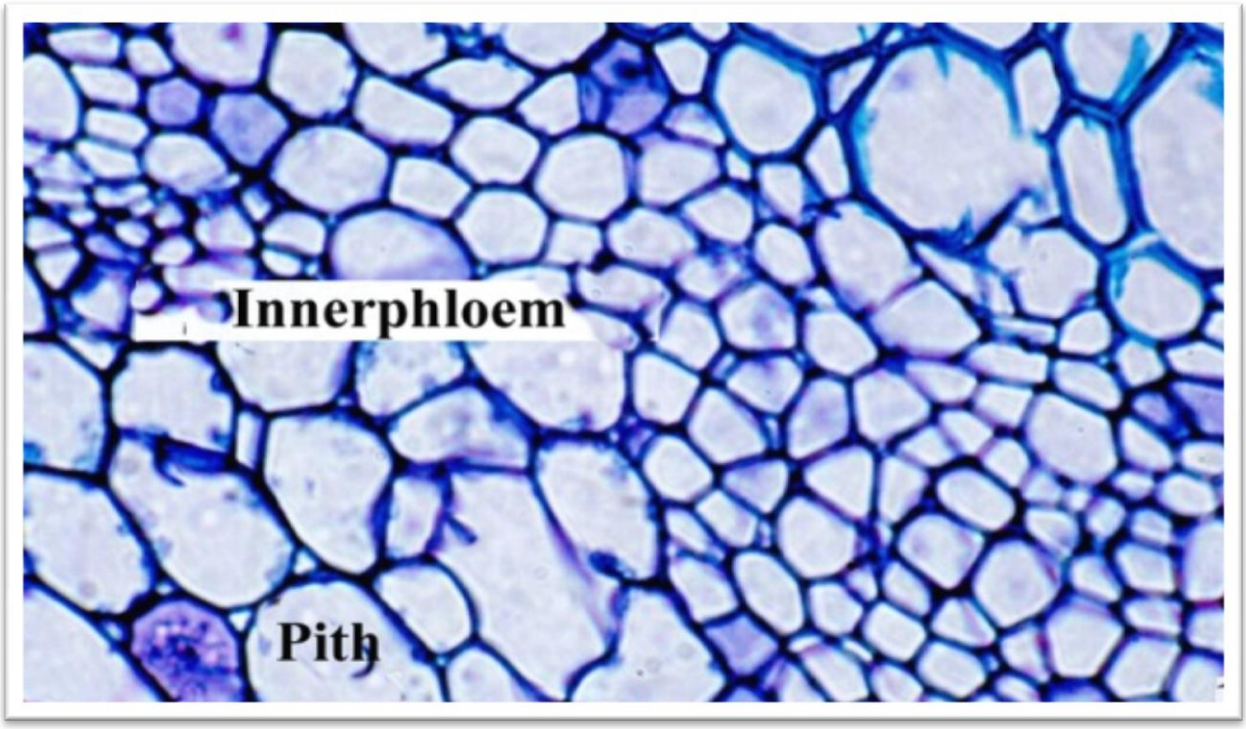
T.S OF STEM INITIAL VIEW SECTOR ENLARGED FIG -12.2



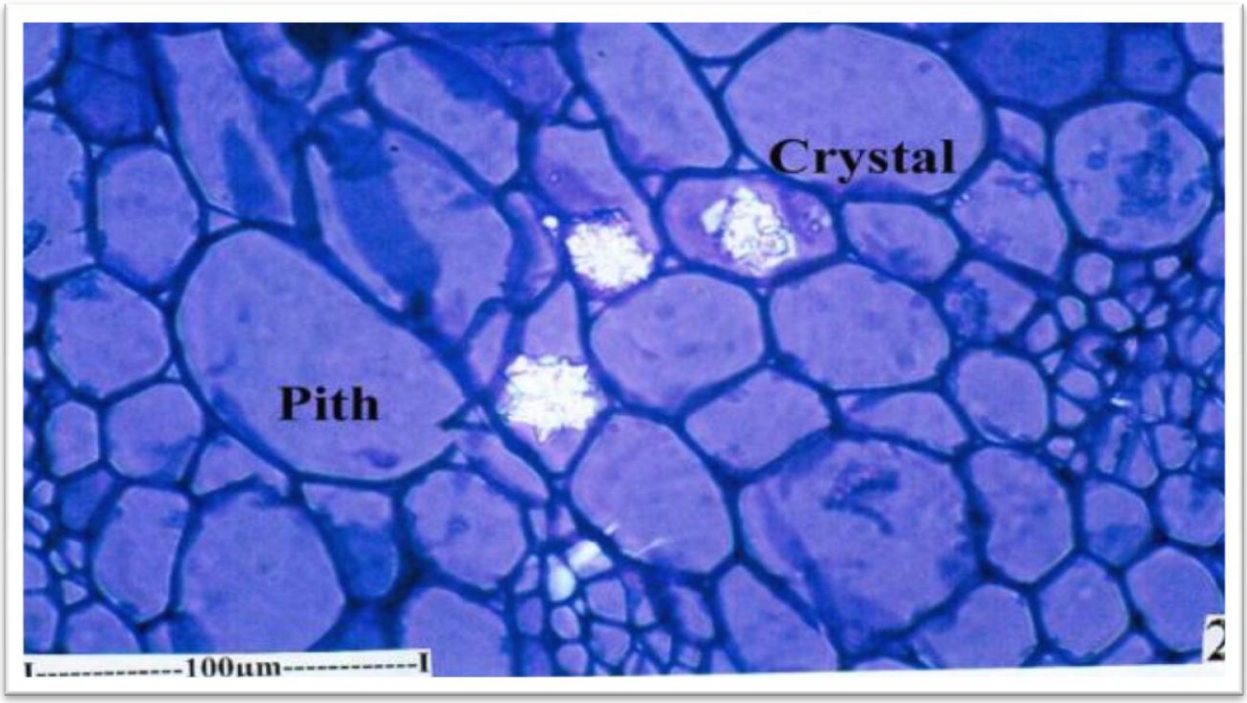
T.S OF STEM VASCULAR CYLINDER ENLARGED FIG -12.3



T.S OF STEM INNER PHLOEM FIG -12.4



CRYSTALS IN THE PITH CELLS (UNDER POLARISED LIGHT) FIG -12.5



REFERENCES

1. K.Narayana iyyer Pharmacognosy of Ayurvedic drug kerala ,university of kerala,1975:vol 5:60

2. B.S.Shah and Qadry J.S. The text book of Pharmacognosy.

   14th edition.(2008-2009) 1, 4.

3 .Vinod D.Rangari "Pharmacognosy and Phytochemistry"1st edition,2002:9.

4 Dr. Pulok . K. Muherjee Quality control of herbal drugs 1st edition Business Horizons,New Delhi,2002:2.

5 C.K.Kokate, A.P.Purohit, S.B.Gokhale "Pharmacognosy" Nirali Prakasham,1998: 94,95

6 Park E.J and Pezzutto,J.M Botanicals in cancer chemoprevention cancer and metastasis Reviews,2002:21:231-255.

7 Rakesh K Sharma, Rajesh Arora Herbal drugs: A twenty first century perspective, Jaypee Brothers medical publishers (P) Ltd,New Delhi,2006:422-423.

8 .Manmeet Kumar, Ausaf Ahmad, Preeti Rawat, Mohammad Faheem Khan, Naila Rasheed, Prasoon Gupta, B. Sathiamoorthy, Gitika Bhatia, Goutam Palit, Rakesh Maurya."Antioxidant flavonoid glycosides from *Evolvulus alsinoides*",Fitoterapia,2010: (81); 234.

9 .Goldmen and Ausiello Cecil Text book of medicine"22 nd edition vol 2;1311.

10 .Trease and Evans" The text book of Pharmacognosy" 16th edition 2009:416,417.

11 .Shukula G,Verma BK. A taxonomic account of the genus Ipomoea in Bunfolkland region (UP Proceedings of the National Academy of Sciences 1990; 60(40): 397-404.

12 Singh V, Gupta PC. A non ionic seed gum from *Ipomoea pes-tigridies*. Int J Pharmacognosy 1995; 3(3): 238-241.

13 .Azam MM, Sherwani MRK. Chemical investigation of some seed oils from acrid zone of Rajasthan. Oriental J Chem 1999; 15(2): 295-300.

14 Sharma DP. Studies on nutritional qualities of *Ipomoea pes-tigiridis* Linn; a fodder plant from Kunvari Ravin land at Morena (MP) India. Asian J Chem 2002; 14(2): 1119-1121.

15 Parveen F, Bhandari MM. Pollen morphology of plants of Indian desert (Convolvulaceae). J Econ & Taxonomic Bot 1982; 3(2): 329-334.

16 Karratela YY, Gill LS. Epidernal morphology and stomatae ontogeny in some West African Convolvulaceae species. Herba Hung 1985; 24(2-3): 11-18.

17 Siddiqui TO, Ahmad J, Sharma N, Botanical character of Kaladana an important unani drug. Hamdrad Medicus 1990; 33(1): 84-85.

18 Sen M, Dey M, Karuri P. Chemical investigation of *Gerbera lanuginosa* Benth (whole plant and leaves of *Ipomoea fistulosa).* Indian Chem. Soc 1979; 56(3): 326-327.

19 Goto T, Konda T, Imagawa H, Miura I, Heavenly blue anthocyanin II:trans -4-O-[6-O-trans-3-O-(β-D-gilucopyranosyl caffeyl)-β-D-glucopyranosyl]-caffeic acid, a novel components in heavenly blue anthocyanin. Tetrahedron Lett.1981; 22(33): 3213-3216.

20 Takagi S, Yamaki M, Masuda K, Nishihama Y, Kubota M, Lu ST. Studies on the constituents of *Ipomoea biloba* forsk. Yakugaku Zasshi 1981; 101(5): 482-484.

21 Dubey P. Khare V, Gupta PC. A new flavonoid glycoside from the leaves of *Ipomoea fistulosa*. Curr Sci 1982; 51(7): 351-352.

22 Schneides JA, Lee J, Naya Y, Nakanishi K, Oba K, Uritani I. The fate of the phytolexin *Ipomoea marone*; Furanoterpenes and butenolides from *Cerato-cystis fimbriata* infected sweet potato. Phytochem 1984; 23(94): 759-764.

23 Harison Da, Kulshreshtha DK, Chemical examination of *Ipomoea dichora*. Fitoterpia 1986: 57(1): 29-32.

24 Wilkinson RE, Hardcastle WS, Mecormic KCS Seed ergot alkaloid contents of *Ipomoea hederifolia Ipomoea quamoclit, Ipomoea coccinea, Ipomoea wrighti.* J. Sci Food, Agric., 1987; 39(4): 335-339.

25 Ono M, Kawasaki T, Miyahara K. Resin glycosides xi operculeins III, IV, IX, XVI, XVII and XVIII, New ether soluble resin glycosides of rhizomes Jalapae Braziliensu (root of *Ipomoea operculata*). Chem & Pharm Bull 1991; 39(10): 2534-2539.

26 Ysrael MC, Waterman PG. Isolation of the antifungal and cytotoxic principles from *Ipomoea muricata*. ASOMPS 1994; 5(14): 175.

27 Yahara S, Yoshimoto M, Yamakawa O. Phenolic compounds from the leaves of *Ipomoea batatas.* Natl Med 2002; 56(3): 121.

28 Pareda-Miranda R, Escalanate–Sanchez E, Escobeda-Martinez C. Characterization of lipophilic pentasaccharides from beach morning glory *Ipomoea pes-caprae* .J Natural Products 2005; 56(12) :1724-1726.

29 Singh V, Pandey M, Srivastava V. Seed mucilage from *Ipomoea dasysperma*. Fitoterapia 2007; 78(3): 268-270.

30 Kappor A, Mahor R, Vaisham Payan N, Gautam N. Antifungal spectral of some petal extracts. Geobies 1981; (2): 66-67.

31 Khare AK, Srivastava MC, Tewa JP, Puri JN, Singh S, Ansari NA. A preliminary study of anti-inflammatory activity of *Ipomoea turpethium* (NISHOTH). Indian Drugs 1982; 19(6): 224-228.

32 Mujumdar AM, Marathe H, Ghaskadbi SM. Pharmacology of *Ipomoea leari*. Indian Drugs 1983; 20(8): 307-310.

33 Da Silvo Filho AA, Correa Lima RMO, De Andrade Chiapett A, Do Nascimento SC, De Souza IA, Demello JF, Veith HJ. Anticancer and antimicrobial glycosides from *Ipomoea bahiensis*. Phytochem 1986; 25(5): 1077-1081.

34 Pongprayoon U. Pharmacologic studies on the Thai medicinal plant *Ipomoea caprae*. Acta pharmaceutica Nordica 1991; 3(3): 186.

35 Reza MS, Khan MOF, Islam MA, Chowdhury AKA. *In vitro* antimicrobial activity of *Ipomoea fistula*. Fitoterapia 1994; 65(5): 465-466

36 .Reynols WF, Yu M, Enriquez RG, Gonzaler H, Leon I, Magos G, Villareal ML. Isolation and characterization of cytotoxic and antibacterial tetrasaccharides, glycosides from *Ipomoea stans*. J Natl Products 1995; 58(11): 1730-1734.

37 Navarro-Ruiz A, Delamora GP, Villanuev Michel MI, Dominguez JR, Bastidas-Ramirez BE, Quezada-Arellano JD, Rui Madrigal B. Anticonvulsant effect of aqueous, hydro alcohol and chloroform extracts from *Ipomoea stans* root in the rat. Phytotheraphy Res 1996; 10(3): 242-244.

38 Matsui T, Kobayashi EM, Sugita F, Terahara N, Matsumoto K. Antihyperglycemic effect of discylated anthocyanin derived from *Ipomea batatas* cultivar ayamurasakri can be achieved through the alpha glucosidase inhibitory action. J Agric Food Chem 2002; 50(25): 7244-7248.

39 Barnes CC, Smalley MK, Kindscha, MKP, Loring K, Sheeley H. Characterization of an tuberculosis resin glycoside from the prairimoplant, *Ipomoea leptophylla*. J Natl Prod 2003; 66(11): 1451-1462.

40 Dhembare AJ and Sangle S. Effect of plant extracts on some important pathogenic bacteria. J Exptl Zool 2003; 6(2): 299-300.

41 Haueza IM, Fonseca ESM, Paulino CA, Haraguchi M, Gorniak SL. Evaluation of immunomodulatory activity of *Ipomoea carnea* on peritonial cells of rats. J Ethnopharmacol 2003; 87: 181- 186

42 Yoshimot M, Kurataa RK, Fuji M, Hou DX, Ikeda K, Dome TY, Osako M. Phenolic composition and radical scavenging activity of sweet potato (*Ipomoea batata)* derived schochu distillery by products treated Koji. Biosci Biotech, Biochem 2004; 68(12); 2477-2483.

43 Prasad KN, Prasad MS, Shivamurthy GR, Aradhya SM. Callus induction from *Ipomea aquatic*(Forsk) leaf and its antioxidant activity. J Biotech 2006; 5(1): 107- 111.

44 Ferreria AA, Amarlal FA, Durate IDG, Oliveria PM, Alves RB, Silveria D, Azevedo AO, Raslan DS, Castro MSA. Antinociceptive effect from *Ipomoea cairica* extract. J Ethnopharmacol 2006; 105(1-2): 148-153.

45 Dattachoudry M, Choudhury R, De B, Paul SB. Physiochemical characterization and anti fungal, CNS depressant activity of certain ethnomedicinal plants of Tripura State, India. Biosci Biotechnol Res Asia 2007; 4(1): 145-148.

46 Sokeng SD, Rokeya B, Kannan FM, Junaida K, Zitech P, Ali L, Ngounou G, Lontsi D, Kamtchouching G. *Ipomoea aquatic* extracts on glucose absorption using a perfused rat intestional preparation..Fitoterapia 2007; 78(7-8): 526-529.

47 Hamsa TP, Kuttan G. Antioxidant activity of *Ipomoea obscura*. Amala Research Bulletin 2007; 27: 24-34.

48 Vimala Y, Elizabeth KM, Srilakshmi Selvi G. Antimicrobial activity of *Ipomoea kentrochules* on pathogenic microorganisms and microbial isolates on spoiled vegetables. Asian J Micro, Biotech & Environmental Sci 2007; (1): 41-44.

49 Rivera IL, Mirron Lopez G, Molina Salinar GM, Herrera–Ruiz M, Alonso-cortes D, Navaneze Vazquez GR, Cos MY, Fernandez S. Trianthinic acids from *Ipomoea tyriantia* and their anti-micobacterial activity, cytotoxicity and effects on the central nervous system. J Natl Prod 2008; 71(10): 1686-1691.

50 Chimkode R, Patil MB, Jallapure SS, Agarwal N, Tripathi R, Tripathi A, Patil NR. Wound healing activity of tuberous root extracts of *Ipomoea batatas*. Adv Pharmacol & Toxicol 2009; 10(3): 69-72.

51 Dass.N., Ray.B, Mahapatra S.K and Pothal R.K. Microbiological potentiality of Ipomoea sepiaria Roxb (Convolvulaceae).International Journal of Research in Pharmaceutical and Biomedical sciences.2011:2(2):500-501.

52 Vijayan.N,Barreto Ida,Dessai Seema, Dhuei Shital, D'Silva Riva and Rodeigues Astrida. Antimicrobial activity of ten common herbs, commonly known as Dashapushpam from kerala, India. Asian Journal of Microbiology Research.2010:4(2):2357-2362.

53 M.Abdul Latif, Sharmina Akter, Shahjahan Kabir, M.Ansar Ali, M.Anwar Hossain and M.Loothfor Rahman.Efficacy of some organic Amendments for the control of ufra Disease of Rice.Bangladesh journal of microbiology.2006:23:118-120.

54 Syed Shayfur Rahman, Md.Mizamur Rahman, Shameem Ara Begum, Mohammad mizamur, Rahman Khan, Md.Mosharef Hossain Bhuiyan. Investigation of Sapindus mukorossi Extracts for Repellency, Insecticidal Activity and plant growth regulatory effect. Journal of Applied Research.2007:3(2):95-101.

55 Mathew, K.M..The Flora of Tamil Nadu Karnatic. Polypetalae. P-Gamopetalae and Mono chlamyda 1983:vol3:688-689.

56 Metcalfe, C. R. and Chalk, L. Anatomy of the Dicotyledons. Clarendon Press, Oxford.1950: Vol I: 276.

57 Metcalfe, C. R. and Chalk, L. Anatomy of the Dicotyledons. Clarendon Press, Oxford.1979:Vol II. 379.

58 O Brien, T.P; Feder,N. and Mc Cull, M.E. Polychromatic staining of plant cell walls by toluidine blue-O.Protoplasma.1964: 59: 364-373.

59 Sass, J. E.Elements of Botanical Micro technique. Mc Graw Hill Book Co; New York.1940:222.

60 Wallis,T. E. Text Book of Pharmacognosy, CBS Publishers and Distributors, Shahdara, Delhi, India.1985.

61 YogaNarasimhan, S. N. Medicinal Plants of India. Tamilnadu Regional Research Institute. Bangalore, India 2000:vol II:715.

62 C.K Kokate practical pharmacognosy, new delhi, vallabh prakashan,4th edition.1994:107,115-120.

63 The Ayurvedic Pharmacopoeia of India 1st edition part-1,of India.1999:vol II :98-101, 128-130

64 Indian Pharmacopoeia controller of publications, Govt. of India, ministry of Health family welfare, Delhi, 1996: A-53, A-54 and A-89.

65 Bently and Drivers Text of Pharmaceutical chemistry 8th edition oxford university press, London,1983: 31.

66 Quality control methods for medicinal plant material world health organization Geneva 1998:33.

67 Evans WC Trease and Evans pharmacognosy 15th edition saunderes London 2002: 193,230,271,336,536.

68 Kokate C.K, Purohit A.P, Gokhale J.B Pharmacognosy Nirali prakasham pune 36th edition2006: 106-109, 271-272, 593-597.

69 I.L. Finar organic chemistry ELBS, London 5th edition.1996:2: 771

70 Gurdeep, Chatwal. R.Organic chemistry Ist edition Himalaya Publishing house Mumbai 2000:2:539.

71 Jain U.K. and Dixit V.K.spectrophtometry estimation of tannins from chyavanprash, Indian drugs2004:469-472.

72 Shingenori, Kumazawa, Masa Taniajughi, Yasuyuki, Suzuki, MasayoSshimura, Mi-sun Kuson and Tsutomu Nakayana J. Agri and food chem..2002:50:373-377.

73 Chang C.C, Yang M.H, Wen H.M, Chern J.C. Estimation of total flavanoids content in propolis by two complimentary colorimetric methods.;JFDA;2002;10;3;178-182.

74 Hildbert Wagner, Sabune Bladt "Plant Drug Analysis" A thin layer chromatography Atlas, 2nd edition.1,2,196,197.

75 J.B.Harborne "Phytochemical methods- A Guide to Modern Techniques of Plant Analysis Springer (India) Private Ltd New Delhi "1998:10,114.

76 Kurt Randerath thin"Thin layer chromatography" verlong chemie Academic press.1966:217.

77 Marinova D, Ribarova F, Antanassova M. Total phenolics and total flavonoid in Bulgarian fruits and vegetables.2005:40:3:255-260.

78 Arunachalam muthuram, Amteshwar singh Jaggl,Nimal singh, Dhandeep singh "Ameliorative effects of amiloride and pralidoxime in chronic constriction Injury and vincristine induced painful neuropathy in rats" European journal of Pharmacology, 2008,587;104.

79 Ross and Wilson "Anatomy and physiology in health and illness" 9th edition:188.
80 Amteshwar singh Jaggi, Nirmal singh, Vivek Jain "Animal Models Neuropathic pain" Fundamental and Clinical Pharmacology.2009:1,2,18.

81. Bing Ling, Alain Eschalier, David Balayssae, Francois coudore, Nicolas Authier "Assessment of Nocicepion in acrylamide induced Neuropathy in rats" , Pain 2005: (119): 104,11

82. Hanaa, H.Ahmed, Gamal A.Elmegeed, El-Sayed M.El- Sayed. Mervat M.Abd-Elhalim, Wafaa Gh. Shousha, Reham W.Shafic. Potent neuropathy role of novel melatonin derivatives for management of central neuropathy induced by acrylamide in rats.European journal medicinal chemistry,2010,45 5452-5459

83. S.S.Agarwal and Dr. M. Paridhavi "Herbal Drug Technology" Universities Press p-607, 611.

84. Eddy N.B, Touch berry C.F, Lieberman J.E, Synthetic analgesics: I. Methadone isomers and derivatives. J. Pharmacol Exp Ther1950: 98: 121-137.

85. Chaplan S.R, Bach F.W, Posrel J.W, Chung J.M, Yaksh J.L.Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Methods1994:53:55-63.

86. Necker R, Hellen R.F,Noxious thermal input from the rat tail, modulation by desending inhibitory influences, Pain1978:4:231-242.

87. Okhawa H, Ohesli N, Yasik, Assay of lipid peroxidation in animal tissue by TBARS, Analyst Biochem.1979:95:351-358.

88. Ellman G.L. Tissue sulfhydryl groups, Arch. Biochem Biophys.1959:82:70-77.

89. Severinghaus J.W, Feru bee J.W, Calcium determination by flame photometry: methods of serum, urine and other fluids. J. Biol Chem.1950:187:621-630.

90. Muthuraman A, DiwanV, JaggiAS, SinghN, SinghD. Ameliorative effects of *Ocimum santum* in sciatic nerve transection-induced neuropathy in rats, J .Ethanopharmacol 2008:120:56-62.

91. Sudoh T, Desai S.P, Haderer A.E, Sudoh S, Gerner P, Anthony D.L, De Girolami U, Wang G.K. Neurologic and histopathologic evaluation after high volume intrathecal amitriptyline. Anaesth. Pain Med2004: 29:434-440

92. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ Protein measurement with Folin phenol reagent. J Biol Chem.1951:193:265-275.

93. Honda K, Casaderus G, Peterson R.B, Perry G, Smith M.A,Oxidative stress and redox active iron in Alzheimer's disease Ann Ny Acad Sci. 2004:1012:179-182.

94. Glass J.D, Culver D.G, Levery A.I, Nash N.R.Very early activation of m-calphin in pheriperal nerve during wallerian degeneration.2002.

95. Carrido S.G, Sensi S.L, Yin H.Z, Weiss J.H Ampa exposures induced mitochondrial Ca (2+) overload and ROS generation in spinal motor neurons in vitro. J. NeuroSci.2000 :20:240-250.

96. Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods.1983: 65, 55-63.

97. Monks, A., et al., Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. Journal of the National Cancer Institute.1991:83, 757-766.

98. Mahesh Chand Meena, Vidya Patni. Isolation and identification of Flavonoid "Quercetin" from Citrullus colocynthis. Asian J.Exp.Sci.,2008:22:137-142.

99. Fatemeh Fathiazad, Abbas Delazar, Royal Amiri. Extraction of flavanoids and quantification of Rutin from waste Tobacco Leaves. Iranian journal of pharmaceutical research.2006:3:222-227.

100. Michele Morsch, Leury G.J.Girardi, Valdir Cechinel-Filho, Christiane Meyre-Silva and Clovis Antonio Rodrigues.Seperation of flavanoids Aleurites moluccana Leaves using Chitosan Modified with Heptaldehye. NIQFAR.2004:59C:649-652.

101. KumikoIshige, David Schubert,Yutaka Sagara Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms.Free Radical Biology & Medicine, 2001,30(4):433-446.

102. Rayqude KS,Kandhare AD Evaluation of ameliorative effet of quercetin in experimental model of alcoholic neuropathy in rats inflammopharmacology 2012 (under printing)

103. Anjaneyulu M ,Chopra K, , Quercetin, a bioflavonoids attenuates thermal hyperalgesia in a mouse model of diabetic neuropathic pain, prog Neuropsycho pharmacol Biol Psychiatry 2003 27(6) 1001-1005.

104. Davis W.Lamson Matthew S, Antioxidants and cancer III;Quercetin Altern Med Rev 2000 5(3);196-208.